Chapter 17

Application of Surface Plasmon Resonance Spectroscopy to Study G-Protein Coupled Receptor Signalling

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Abstract

The G-protein coupled receptor rhodopsin is a classical example of a seven transmembrane helix receptor; it is photoexcited and transmits this light signal to a G-protein mediated cascade. Many components of this receptor-triggered cascade can be purified in their native forms from natural sources making this system most suitable for biophysical studies. A central aspect of cellular signal transduction routes is to understand protein–protein interactions in a quantitative way. Surface plasmon resonance (SPR) spectroscopy is a biosensor-based technique that allows investigating molecular interactions by determining kinetic parameters. We here show how dark-adapted rhodopsin can be immobilized on the sensor chip surface. A laser device implemented in the SPR system allowed us to trigger light-induced conformational changes in rhodopsin and to monitor light-dependent binding of the photoreceptor cell G-protein transducin to rhodopsin. The sensor chip surface can be regenerated and used for several rounds of interaction analysis. Furthermore, illuminated rhodopsin can be regenerated by applying 9-*cis*-retinal on the sensor chip surface.

Key words: Surface plasmon resonance, rhodopsin, G-protein coupled receptor, G-protein.

1. Introduction

The superfamily of G-protein coupled receptors (GPCR) is involved in the transmission of hormones, neurotransmitters or sensory signals. These diverse signals are further processed by specific receptor coupling to heterotrimeric G-proteins that can interact and regulate effector molecules like adenylate cyclases, phosphodiesterases and ion channels (1–3). A well-understood example of a GPCR pathway is the light-triggered enzyme cascade of vertebrate photoreceptor cells (4, 5). Absorption of single

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photons by the visual pigment rhodopsin is sufficient to produce a photoresponse from a rod cell. This remarkable performance is achieved by a precise arrangement of interacting proteins that leads to a highly amplified hydrolysis of the second messenger guanosine-3′,5′-cyclic monophosphate (cGMP). The heterotrimeric G-protein transducin (G_t) is activated by the light-activated form of rhodopsin, Meta II, which catalyses a GDP/GTP-exchange at the G_t α-subunit. The effector molecule of G_t is the cGMP-specific phosphodiesterase PDE6 that hydrolyses its substrate cGMP with a high turnover number. The downstream target of cGMP is a cyclic nucleotide-gated channel located in the plasma membrane of the photoreceptor cell, which is opened by cGMP and is closed upon hydrolysis of cGMP causing a halt of cation influx and a hyperpolarization of the cell (6).

Investigation of protein–protein interactions that involve membrane-bound proteins like receptor molecules is a challenging task, because for many experimental approaches the receptor needs to be extracted from the membrane while maintaining its structural and functional integrity. The application of SPR spectroscopy for the study of membrane proteins has recently attained increasing interest (7–12). SPR is an optical phenomenon that depends on an ultra-thin metal layer, which is located at the interface of two media of different refractive indices (13). In commercial systems like Biacore© these two media are a prism and a compartment containing an aqueous buffer. When light hits the prism under the angle of total internal reflection, the electric field of the photons extends beyond the reflecting interface into the medium of lower refractive index (e.g. compartment with an aqueous buffer) thereby creating a so-called evanescent electric field. Depending on the conditions of the measuring system a resonance phenomenon is observed that reduces the intensity of the reflected light beam. This phenomenon is highly sensitive to the geometry of the optical system, the wavelength of the incident light, the thickness of the metal layer and the refractive index of the medium. It therefore allows detecting any changes in refractive index close to the sensor chip surface, when all other parameters are kept constant during an analysis.

Protein–protein interaction studies using SPR require the immobilization of one binding partner, while the other partner is supplied in a buffer stream flowing over the sensor surface. Membrane receptor molecules could be immobilized via binding to an immobilized lectin, when they harbour a carbohydrate moiety. We made use of the mannose-rich oligosaccharide chains in rhodopsin to specifically bind the receptor via its amino-terminal part to immobilized concanavalin A (ConA) on the sensor surface (10, 11). This arrangement exposes the loops and the carboxy terminus of rhodopsin to the buffer flow allowing the supply of

interacting partners that bind to the cytoplasmic regions of rhodopsin. In order to trigger rhodopsin activation by light directly on the sensor chip surface, we integrated a laser system into the commercial SPR device. Flashes of light or constant illumination are channelled onto the sensor surface from the light source (laser) by an optic fibre (11).

2. Materials

2.1. Preparation of Bovine Rod Outer Segment Membranes and Proteins

1. Hypotonic buffer: 5 mM Tris–HCl, pH 7.5, 0.5 mM $MgCl_2$, 0.4 mM EDTA, 1 mM DTT. Store at 4°C.
2. GTP (Sigma-Aldrich) solution: 20 μM GTP in hypotonic buffer (see above).
3. Resuspension buffer: 20 mM Tris–HCl, pH 7.5.
4. CHAPS stock solution: 250 mM 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate (Serva) in H_2O. Store at room temperature.
5. Potter homogenizer.
6. PD-10 column (GE Healthcare).
7. Liquid nitrogen.

2.2. Immobilization of ConA and Rhodopsin

1. CM5 sensor chips were from Biacore AB (GE Healthcare).
2. NHS solution: 50 mM *N*-hydroxysuccinimide dissolved in H_2O. Prepare a stock and store aliquots at –20°C.
3. EDC solution: 200 mM *N*-ethyl-*N′*-[(dimethylamino)propyl]carbodiimide dissolved in H_2O. Prepare a stock and store aliquots at –20°C.
4. Ethanolamine solution: 1 M ethanolamine hydrochloride, pH 8.5.
5. Concanavalin A stock solution: 5 mg/mL ConA (from *Canavalia ensiformis*, type IV, Sigma-Aldrich) in 100 mM sodium acetate, pH 5.0. Store at –80°C.
6. MES-buffer: 50 mM 2-(*N*-morpholino)ethanesulfonic acid (MES), pH 6.0, 1 mM $CaCl_2$ and 1 mM $MnCl_2$. Store at 4°C.

2.3. SPR Measurements

1. Recordings were performed with a Biacore 2000.
2. Running buffer: 50 mM 3-morpholinopropane-1-sulfonic acid (MOPS), pH 7.5, 150 mM NaCl, 3 mM $MgSO_4$, 10 μM $CaCl_2$, 10 μM $MnCl_2$. Filtrate the running buffer before use (0.22 μm filter, Millipore). Check running buffer daily for particles. Repeat filtration, if necessary.

3. 200 μM GTP solution: prepared from a 50 mM stock solution by dilution with running buffer.
4. Diode-pumped Nd:YAG solid state laser (monochromatic light of $\lambda = 532$ nm, model LL-11 qps) was from Laser-Compact Co. Ltd. The optic fibre had a diameter of 0.5 mm.
5. BIAevaluation 4.1 software.

2.4. Regeneration of Rhodopsin and of Rhodopsin-Coated Surfaces

1. Solution of 9-*cis*-retinal (Sigma-Aldrich): 100 mM stock in dimethyl sulfoxide (DMSO). Store aliquots in light-protected vials at –80°C.
2. Running buffer for regeneration procedure is the same as for SPR measurements.
3. Regeneration solution I: 100 mM methyl α-D-mannopyranoside (Sigma-Aldrich) supplemented with 30 mM *n*-octyl-β-glucoside (Sigma-Aldrich).
4. Regeneration solution II: 6 M urea.
5. Filter all solutions except the 9-*cis*-retinal stock before use in the Biacore device.

3. Methods

The starting material for the preparation of rod outer segment (ROS) membranes, rhodopsin and transducin, is a highly purified sample of rod outer segments. In particular the preparation of bovine rod outer segments from isolated retinae is a well-established procedure and outlined in detail in several publications (e.g. 14, 15). It is not described here. ROS can be stored at –80°C under protection from light for many years without loss of biological activity of most key proteins. All handling of ROS must occur under very dim red light. It is also important to operate the Biacore system in a dark room. While routine steps like washing of the system, activation of the sensor chip surface and immobilization of ConA can be performed under daylight conditions, it is necessary to exclude any light from the rhodopsin sample. A summarizing scheme of all steps is displayed in **Fig. 17.1**.

3.1. Preparation of Rhodopsin and G_t for SPR Interaction Studies

1. For preparation of washed dark ROS membranes, take a sample of bovine ROS (1 mL containing rhodopsin at a concentration of 5–10 mg/mL) and add 5 mL of hypotonic buffer. Homogenize by vortexing and centrifuge (300,000*g*, 15 min). Discard the supernatant and repeat this washing step three times. Keep samples cool at 4°C all the time.

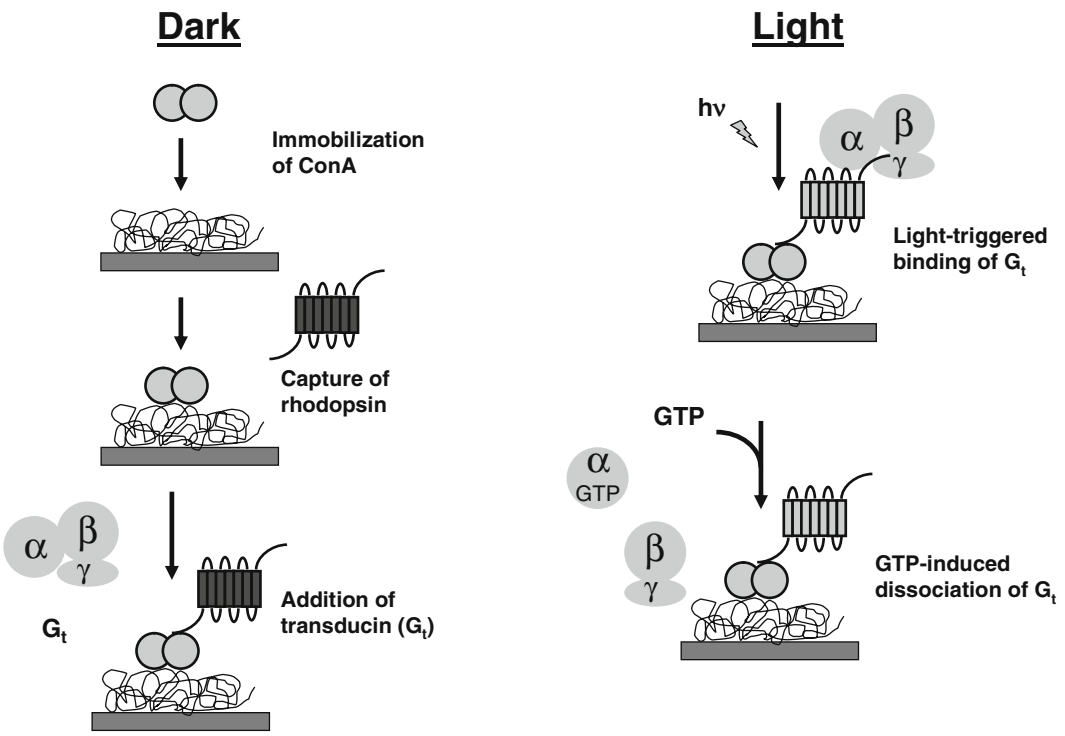

Fig. 17.1. Summary of all steps performed on the SPR sensor chip involving rhodopsin. *Dark*: ConA is immobilized via its free amino groups on a surface with activated carboxy groups. Rhodopsin is captured from the flowing buffer solution by interaction of its carbohydrate moiety with ConA. This allows a site-directed immobilization of rhodopsin on the amino terminus leaving the carboxy-terminal region (i.e. cytoplasmic parts) free for protein–protein interaction analysis. Injection of G_t into the buffer flow leads to a negligible binding to the surface. *Light* : illumination of the sensor chip surface by a laser light triggers interaction of G_t with rhodopsin. Addition of GTP induces the rapid dissociation of G_t from rhodopsin.

Suspend the final pellet of washed membranes in resuspension buffer and store it in aliquots at –80°C.

2. For the isolation of G_t (16, 17) take 1 mL of bovine ROS (rhodopsin concentration 5–10 mg/mL) and illuminate it with normal white light for 20 min on ice. Then add 5 mL of hypotonic buffer and homogenize the suspension of ROS by using a Potter homogenizer. Avoid foaming of the sample. Centrifuge at 300,000*g* for 15 min. Discard supernatant and repeat this washing step five times. Elute G_t from the membranes by incubation of ROS membranes for 5 min with 2.5 mL of 20 μM GTP in hypotonic buffer. Centrifuge once more (300,000*g*, 15 min) and then use a PD-10 column to remove GTP. Removal of GTP is important to observe light-dependent G_t–rhodopsin interaction. In order to minimize bulk refractive index changes during the injection of G_t samples into the microfluidic system of the Biacore device equilibrate the PD-10 column with running buffer. Use liquid N_2

to freeze the final sample of G_t and store aliquots at –80°C until use (*see* **Note 1**).

3. Measure protein concentration of G_t by a Coomassie blue assay (18) and of the rhodopsin preparation by the absorbance at 498 nm ($\varepsilon_{498} = 42{,}000\ M^{-1}\ cm^{-1}$). Analyse samples by sodium dodecyl sulfate polyacrylamide gel electrophoresis (19).
4. Rhodopsin is solubilized from a suspension of washed ROS membranes (50–100 μg of rhodopsin) by addition of CHAPS to reach a final concentration of 25 mM in resuspension buffer. After 30 min of gentle shaking at 4°C, nonsolubilized material was sedimented by centrifugation at 18,000*g* for 15 min. Take the supernatant, protect from light and store on ice until use. The resulting preparation is highly enriched in rhodopsin (11) and can be used without further purification for immobilization on the sensor chip surface (*see* **Note 2**).

3.2. Immobilization of Proteins on the SPR Chip Surface

1. CM5 sensor chips (Biacore) contain a carboxy-methylated matrix that can be activated by a fresh mixture of *N*-hydroxysuccinimide (NHS) and *N*-ethyl-*N*′-[(dimethylamino)propyl]carbodiimide (EDC) to allow the covalent coupling of proteins via its free amino groups. Set the flow rate to 5 μL/min. In order to couple ConA activate the matrix first by 35 μL of 50 mM NHS and 200 mM EDC.
2. Inject 35 μL of 0.1 mg/mL ConA in 100 mM sodium acetate buffer, pH 5.0.
3. Activated carboxy groups that have not been reacted with ConA need to be deactivated before start of any interaction analysis. Therefore, deactivate the surface by injection of 35 μL of ethanolamine solution.
4. Take a freshly prepared stock solution of rhodopsin in CHAPS (*see* step 4 of **Section 3.1**). Just before immobilization, dilute the solution with MES-buffer fivefold (this buffer contains Ca^{2+} and Mn^{2+} cations which are essential for carbohydrate binding by ConA) and set the flow rate to 2 μL/min. Inject 50 μL of solubilized rhodopsin (4–400 μg/mL) (*see* **Note 3**).
5. When the injection and immobilization are finished, set the flow rate to 30 μL/min. Wash the system with running buffer until a stable baseline is recorded. This will take at least 30 min. A sensorgram displaying all steps of activation, coupling, deactivation and binding is shown in **Fig. 17.2**.
6. Calculate the amount of immobilized ConA and immobilized rhodopsin by measuring the difference in resonance

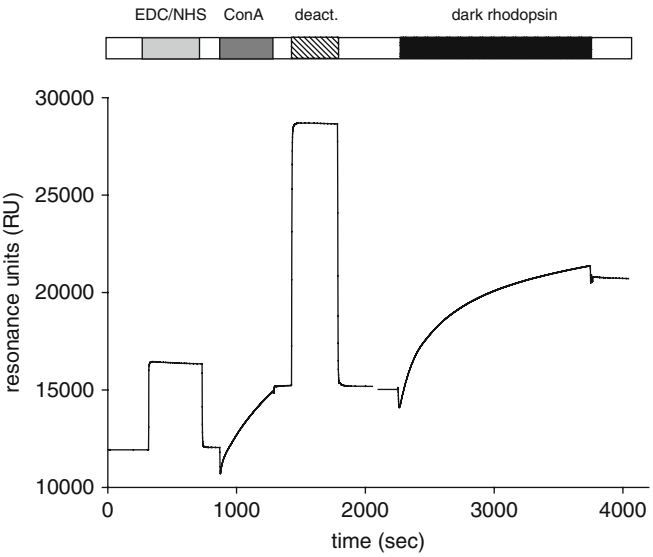

Fig. 17.2. Sensorgram displaying the immobilization of ConA and rhodopsin. A mixture of EDC and NHS activates the carboxy-methylated matrix of a CM5 sensor chip for subsequent coupling of free amino groups (*light grey bar*). Injection of ConA (*dark grey bar*) leads to a reaction of free amino groups in ConA with activated carboxy groups on the surface. Deactivation of nonreacted carboxy groups is performed by flushing the system with ethanolamine (*hatched bar*). Injected rhodopsin is captured by ConA (*dark bar*).

units (RU) before and after injection of ConA and rhodopsin. A change of 1 RU corresponds to an amount of 1 pg protein per mm^2.

3.3. Interaction of G_t with Immobilized Rhodopsin

The analysis of a G-protein/receptor interacting pair has to look for the G-protein binding step that is triggered by ligand activation of the receptor and the GTP-induced dissociation of the G-protein from its receptor. In the case of native rhodopsin 11-*cis*-retinal is a covalently bound ligand located in a receptor-binding pocket. Light triggers the conversion of the inactive ligand to an active form leading finally to the catalytically active Meta II state of rhodopsin. Commercial SPR systems are not equipped with an external light source to illuminate the sensor chip surface. The system used in this study was modified by integration of an optical fibre (diameter = 0.5 mm) that was placed directly on the interfluid cartridge (IFC) device from Biacore by fixing it with scotch tape. The fibre was connected to a diode-pumped Nd:YAG solid state laser (model LL-11 qps; Laser-Compact Co. Ltd.), which allows to illuminate the sensor chip surface with light of λ = 532 nm (*see* **Note 4**).

1. Check how the illumination of the sensor chip surface by the laser will disturb the performance of the system, in particular whether artefact signals are produced. For this

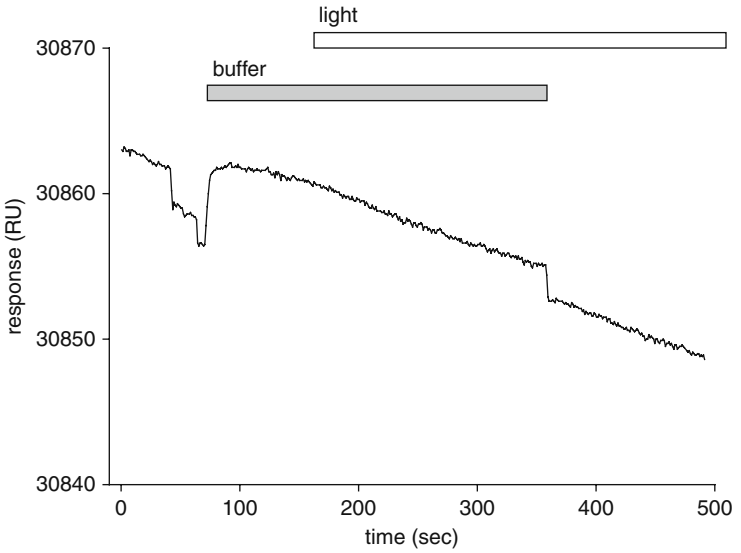

Fig. 17.3. Control recording showing the influence of the laser illumination on the resonance signal. Running buffer is injected into running buffer that is flowing over a ConA-coated surface. Injection is indicated by the *grey bar*. The laser light is switched on as indicated by the *white bar*. Disturbance of the system is very low in the order of maximal 1–2 RU, which is similar to the noise of the system.

purpose run the system without immobilized rhodopsin or after rhodopsin has been bleached by laser light. The Biacore signal should not be affected by light from the laser light source. *See* for example **Fig. 17.3**.

2. Set the flow rate to 5 μL/min and keep the system in the dark. Inject 35 μL of purified G_t (*see* step 2 of **Section 3.1**) at different concentrations (0.0175–0.7 μM).
3. Observe possible dark (or nonspecific) binding of G_t to the surface (*see* **Note 5**). Let the signal reach a plateau (usually within 1 min) and start illumination by switching on the laser. Use either flash illumination or constant illumination depending on the aim of the experiment.
4. When the injection of G_t is finished, wash the flow cell for 5 min with running buffer. Initiate dissociation of G_t from bleached rhodopsin by application of 200 μM GTP (inject 25 μL). The sequence of binding and dissociation events can be seen in **Fig. 17.4**.

3.4. Regeneration of Rhodopsin and of a Rhodopsin-Coated Sensor Chip Surface

Due to a limited supply of 11-*cis*-retinal one can also use the commercially available 9-*cis*-retinal to regenerate rhodopsin. Regeneration can be performed directly on the chip surface by injecting 9-*cis*-retinal into the flow cell. Alternatively, the surface can be regenerated by removing rhodopsin from ConA. This can be achieved by injection of the competing carbohydrate

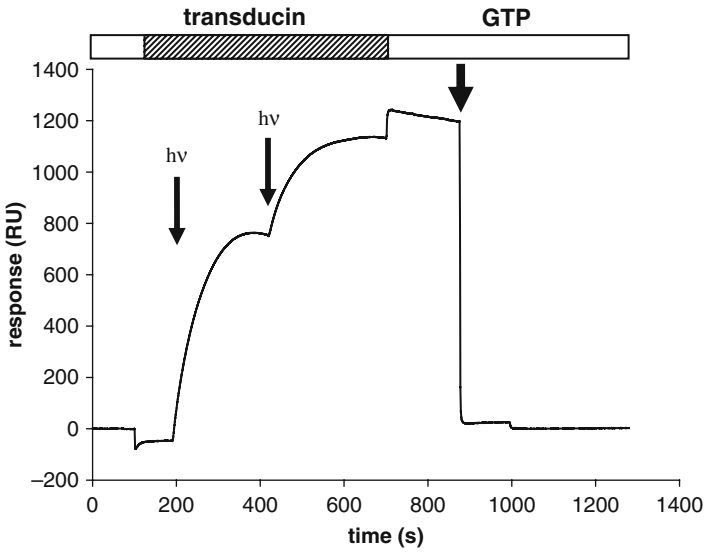

Fig. 17.4. Light-triggered binding of G_t to rhodopsin. G_t is injected into the flow cell (*hatched bar*) under dark conditions, which causes first a small decrease of the resonance signal due to bulk refractive index changes and then a small increase indicating binding of G_t to the surface. Illumination of the system is indicated by two *thin arrows*. Bleaching of rhodopsin triggers binding of G_t as is seen by the positive changes in RU. Running buffer without G_t causes only slow dissociation of G_t (see small change in RU from the end of G_t injection to the third arrow). GTP leads to complete dissociation of G_t from rhodopsin (*thick arrow*).

methyl-α-D-mannopyranoside in a detergent mix and by injection of urea (*see* **Note 6**).

1. Set the flow rate to 5 μL/min. Inject consequently 35 μL of regeneration solution I and 5 μL of regeneration solution II. Wait until the baseline is stable and measure the RU. Compare the value with the starting RU before addition of rhodopsin to the system (*see* **Note 7**).
2. Alternatively, regenerate rhodopsin online by applying 9-*cis*-retinal in the dark. Inject 120 μL of 10 μM solution of 9-*cis*-retinal (diluted from 100 mM stock solution by running buffer just before injection) at low flow rate (1 μL/min) (*see* **Note 7**).

4. Notes

1. G_t is a very labile protein. Therefore, freeze it only once in liquid nitrogen (shock freezing) directly after purification, when the prepared sample is not used immediately. Do not freeze an aliquot of G_t again, when it has been thawed and

used for experiments. It is also important to remove completely GTP before starting the binding experiments with rhodopsin. That is necessary, because in the presence of an even negligible amount of remaining GTP (as well as GDP) in the sample we observed low or no G_t binding to rhodopsin. Instead of a purified sample of G_t it is also possible to use an extract enriched in G_t that could be obtained according to 17 and as described above (*see* step 2 of **Section 3.1**). Such a preparation also contains a small amount of photoreceptor-specific PDE6 and some other retinal proteins in low quantities. However, the presence of these proteins does not interfere with the G_t–rhodopsin interaction monitored by SPR on a Biacore sensor chip.

2. Best results were obtained with a freshly solubilized and prepared sample of rhodopsin on the day of the experiment. The lifetime of immobilized dark-adapted rhodopsin on a sensor chip is surprisingly long (several days), but there are some factors that may decrease its functional properties. For instance, long exposure of rhodopsin deposited on sensor chip surfaces to detergents may cause loss of receptor function. Therefore we usually do not include detergent to Biacore running buffer, but we assume that a mixture of lipid and detergent molecules is interacting tightly with the hydrophobic parts of rhodopsin forming probably a protecting shelf.
3. We found that CHAPS is the best choice among other detergents that are frequently used for rhodopsin solubilization. It probably helps in maintaining the structural integrity during subsequent experiments on the sensor chip surface. When other detergents were used during solubilization (for example, dodecyl-β-D-maltoside, *n*-octyl-β-D-glycoside or Triton X-100), rhodopsin was less stable and interaction with G_t was less efficient.
4. As a light source for this purpose we used a laser of $\lambda = 532$ nm connected to a thin and flexible optic fibre. The fibre can be connected with the Biacore system without disturbing the SPR signal. It was fixed on the interfluid cartridge (IFC) in a distance of 1 cm away from the capillary system on the sensor chip surface (**Fig. 17.5**). It is important to check that the surface is homogeneously illuminated. Filters can be used to attenuate the light intensity. Alternatively, one can use a strong source of white light instead of a laser.
5. Injection of G_t over dark-adapted rhodopsin can give small positive changes in RU, which originates from either nonspecific interaction of G_t with the sensor chip dextran matrix or binding of G_t to pre-bleached rhodopsin. We could not exclude the presence of small amounts of illuminated

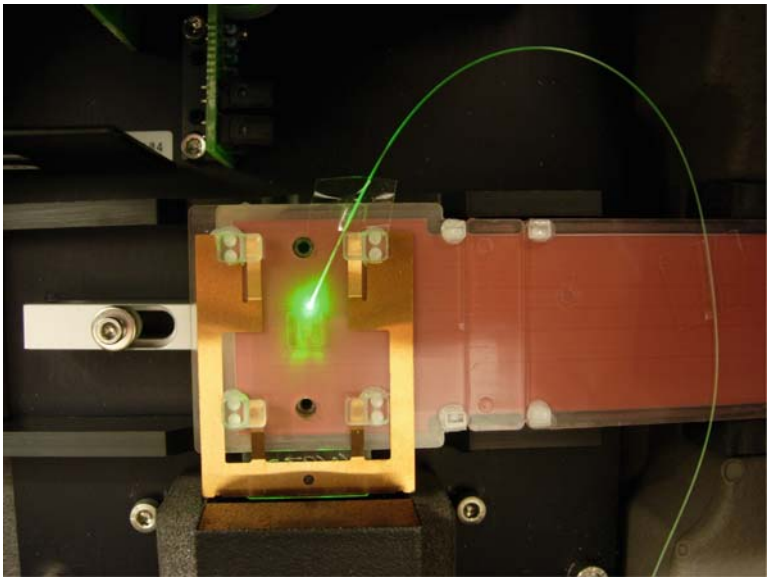

Fig. 17.5. Connection of an optic fibre with the IFC of the Biacore system. The *green light* of the laser is focussed via the optic fibre on the capillary system. The sensor chip has been removed for better visualization.

rhodopsin in a sample of dark-adapted rhodopsin due to the extreme light sensitivity of the receptor. Depending on the purpose of the experiment dark binding of G_t could be subtracted using a corresponding control injection.

6. We observed that the ConA-coated surface has a limited lifetime and we used the surface for approximately 20–30 cycles of immobilization–regeneration. Afterwards, it is advisable to prepare a new surface of ConA. The maximal binding capacity for rhodopsin was only achieved with a fresh surface of ConA. During subsequent cycles of regeneration and immobilization the amount of immobilized rhodopsin decreased steadily indicating a loss of effective ConA-binding sites.
7. Regeneration of rhodopsin by 9-*cis*-retinal was never complete and in the best cases gave 90% recovery of original rhodopsin activity towards G_t binding.

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References

1. Helmreich, E. J. M., and Hofmann, K. P. (1996) Structure and function of proteins in G-protein-coupled signal transfer. *Biochim. Biophys. Acta* **1286**, 285–322.
2. Pierce, K. L., Premont, R. T., and Lefkowitz, R. J. (2002) Seven-transmembrane receptors. *Mol. Cell Biol.* **3**, 639–50.
3. Oldham, W. M., and Hamm, H. E. (2008) Heterotrimeric G Protein activation by G-protein-coupled receptors. *Nature Rev. Mol. Cell. Biol.* **9**, 60–71.
4. Arshavsky, V. Y., Lamb, T. D., and Pugh, E. N. Jr. (2002) G proteins and phototransduction. *Annu. Rev. Physiol.* **64**, 153–87.
5. Filipek, S., Stenkamp, R. E., Teller, D. C., and Palczewski, K. (2003) G protein–coupled receptor rhodopsin: a prospectus. *Annu. Rev. Physiol.* **65**, 851–79.
6. Kaupp, U. B., and Seifert, R. (2002) Cyclic nucleotide-gated ion channels. *Physiol. Rev.* **82**, 769–824.
7. Salamon, Z., Cowell, S., Varga, E., Yamamura, H. I., Hruby, V. J., and Tollin, G. (2000) Plasmon resonance studies of agonist/antagonist binding to the human δ–opioid receptor: new structural insights into receptor ligand interactions. *Biophys. J.* **79**, 2463–74.
8. Neumann, L., Wohland, T., Whelan, R. J., Zare, R. N., and Kobilka, B. K. (2002) Functional immobilization of a ligand-activated G-protein-coupled receptor. *ChemBioChem.* **3**, 993–98.
9. Bieri, C., Ernst, O. P., Heyse, S., Hofmann, K. P., and Vogel, H. (1999) Micropatterned immobilization of a G-protein-coupled receptor and direct detection of G protein activation. *Nat. Biotechnol.* **17**, 1105–08.
10. Clark, W. A., Jian, X., Chen, L., and Northup, J. K. (2001) Independent and synergistic interaction of retinal G-protein subunits with bovine rhodopsin measured by surface plasmon resonance. *Biochem. J.* **358**, 389–97.
11. Komolov, K. E., Senin, I. I., Philippov, P. P., and Koch, K.-W. (2006) Surface Plasmon Resonance study of G protein/receptor coupling in a lipid bilayer-free system. *Anal. Chem.* **78**, 1228–34.
12. Vidic, J., Pla-Roca, M., Grosclaude, J., Persuy, M.-A., Monnerie, R., Caballero, D., Errachid, A., Hou, Y., Jaffrezic-Reanult, N., Salesse, R., Pajot-Augy, E. „ and Samitier, J. (2007) Gold surface functionalization and patterning for specific immobilization of olfactory receptors carried by nanosomes. *Anal. Chem.* **79**, 3280–90.
13. Schuck, P. (1997) Use of surface plasmon resonance to probe the equilibrium and dynamic aspects of interactions between biological macromolecules. *Annu. Rev. Biophys. Struct.* **26**, 541–66.
14. Schnetkamp, P. P. M., and Daemen, F. J. M. (1982) Isolation and characterization of osmotically sealed bovine rod outer segments. *Methods Enzymol.* **81**, 110–16.
15. Koch, K.-W., Lambrecht, H.-G., Haberecht, M., Redburn, D., and Schmidt, H. H. (1994) Functional coupling of a Ca^{2+}/calmodulin-dependent nitric oxide synthase and a soluble guanylyl cyclase in vertebrate photoreceptor cells. *EMBO J.* **13**, 3312–20.
16. Kühn, H. (1981) Interactions of rod cell protein with the disk membrane: influence of light, ionic strength, and nucleotides. *Curr. Top. Membr. Transp.* **15**, 171–201.
17. Bornancin, F., Pfister, C., and Chabre, M. (1989) The transitory complex between photoexcited rhodopsin and transducin. Reciprocal interaction between the retinal site in rhodopsin and the nucleotide site in transducin. *Eur. J. Biochem.* **184**, 687–98.
18. Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–54.
19. Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–85.