Supporting Information

Controlling Transmembrane Protein Concentration and Orientation in Supported Lipid Bilayers

P. Bao[1], M. L. Cartron[2], K. H. Sheikh[3], B. R. G. Johnson[1], C. N. Hunter[2],

S. D. Evans[1*]

[1]School of Physics and Astronomy, University of Leeds, Leeds, LS2 9JT, UK

[2]Department of Molecular Biology & Biotechnology, University of Sheffield, Sheffield, S10 2TH, UK

[3]School of Biomedical Sciences, University of Leeds, Leeds, LS2 9JT, UK

*Corresponding author: Prof. Steve Evans; Email: S.D.Evans@leeds.ac.uk

Table of Content:

Figure S1. The FRAP results for pR-Alexa488 in a DOPC lipid bilayer formed on glass substrate. The recovery curve for FRAP of pR-Alexa488 (hollow dots: experiment results; red line: fitting curve). Inset: Fluorescence images of pR-Alexa488 containing SLB, left) immediately after photobleaching and right) 700 s after photobleaching.

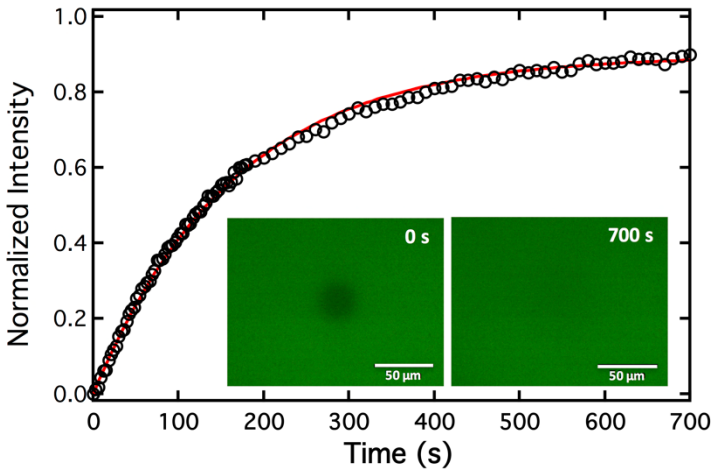


Figure S2. The AFM image of pR-Alexa488 molecules in the DOPC lipid bilayer on glass substrate. The pR molecules were observed in the lipid bilayer formed on glass. However, the density of the particles observed was much lower than that observed in lipid bilayer formed on mica due to that most of the pR is mobile. (a) 1 μm scan; (b) 5 μm scan.

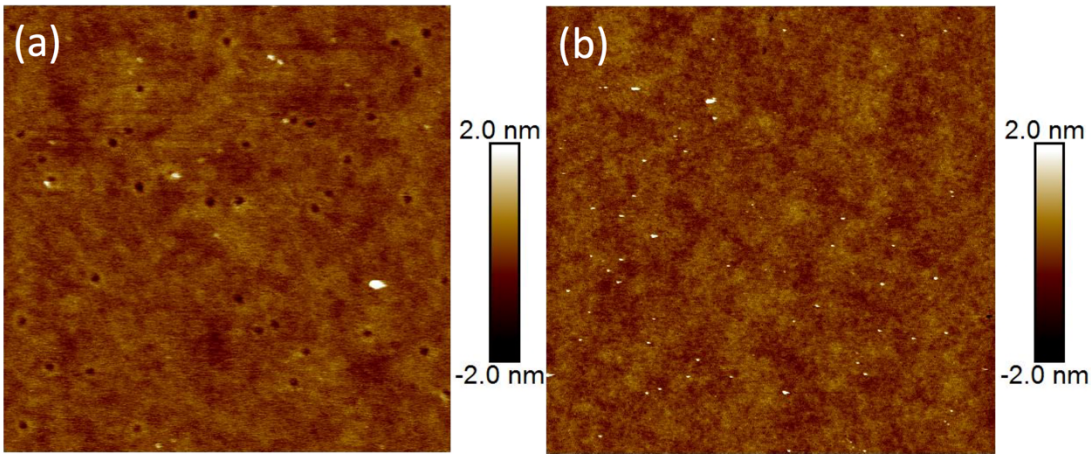


Figure S3. (a) The AFM image of pure DOPC lipid bilayer on glass substrate. Nano-sized holes were observed in the lipid bilayer (1 μm scan); (b) The AFM image of bare glass substrate (1 μm scan); (c) the line profile along the dotted line in (a); (d) the line profile along the dotted line in (b).

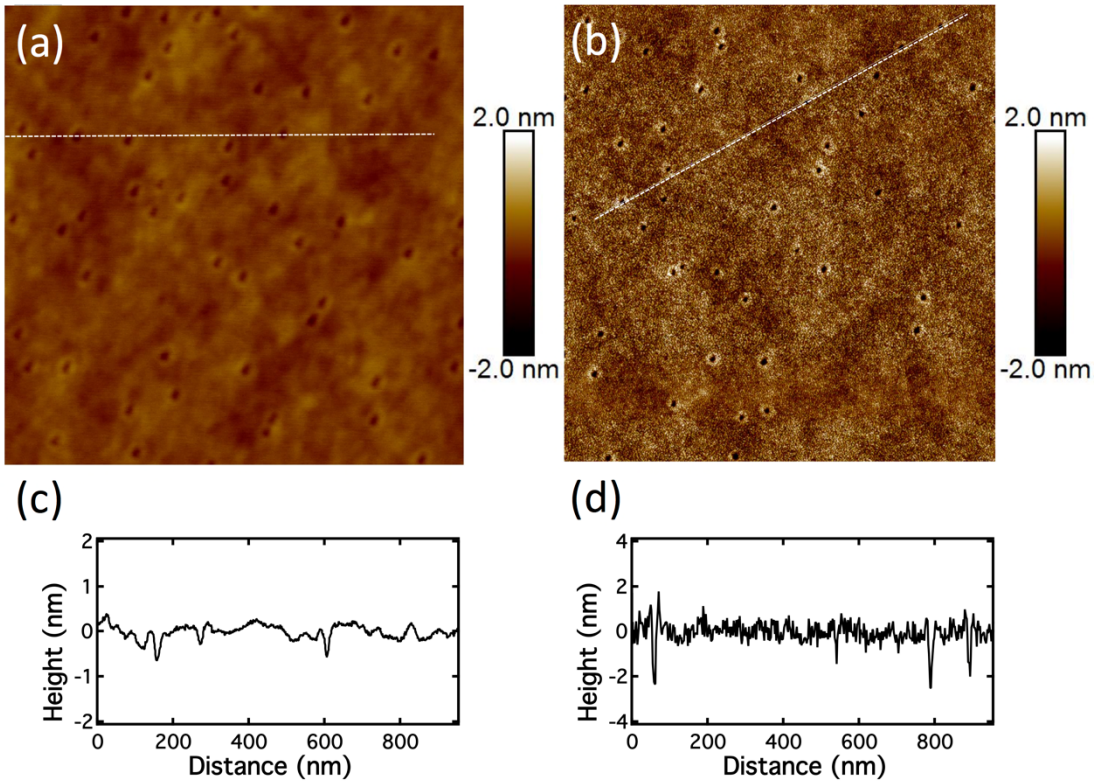


Figure S4. The AFM image of pR-Alexa488 molecules in DOPC lipid bilayer formed on mica. (a) Height image (1 μm scan); (b) height image (1 μm scan) and particle labeling; (c) high resolution image of pR (166 nm scan); (d) height image (2 μm scan); (e) the histogram and two-peaks fitting of the center heights of pRs in lipid bilayer on mica suggested two populations of pRs existing in lipid bilayer, which might represent proteins in two possible orientations or proteins with/without fluorescent label. Peak 1: position = 0.8 nm; FWHM = 0.5 nm; area% = 49%. Peak 2: position = 1.3 nm; FWHM = 1.0 nm; area% = 51%.

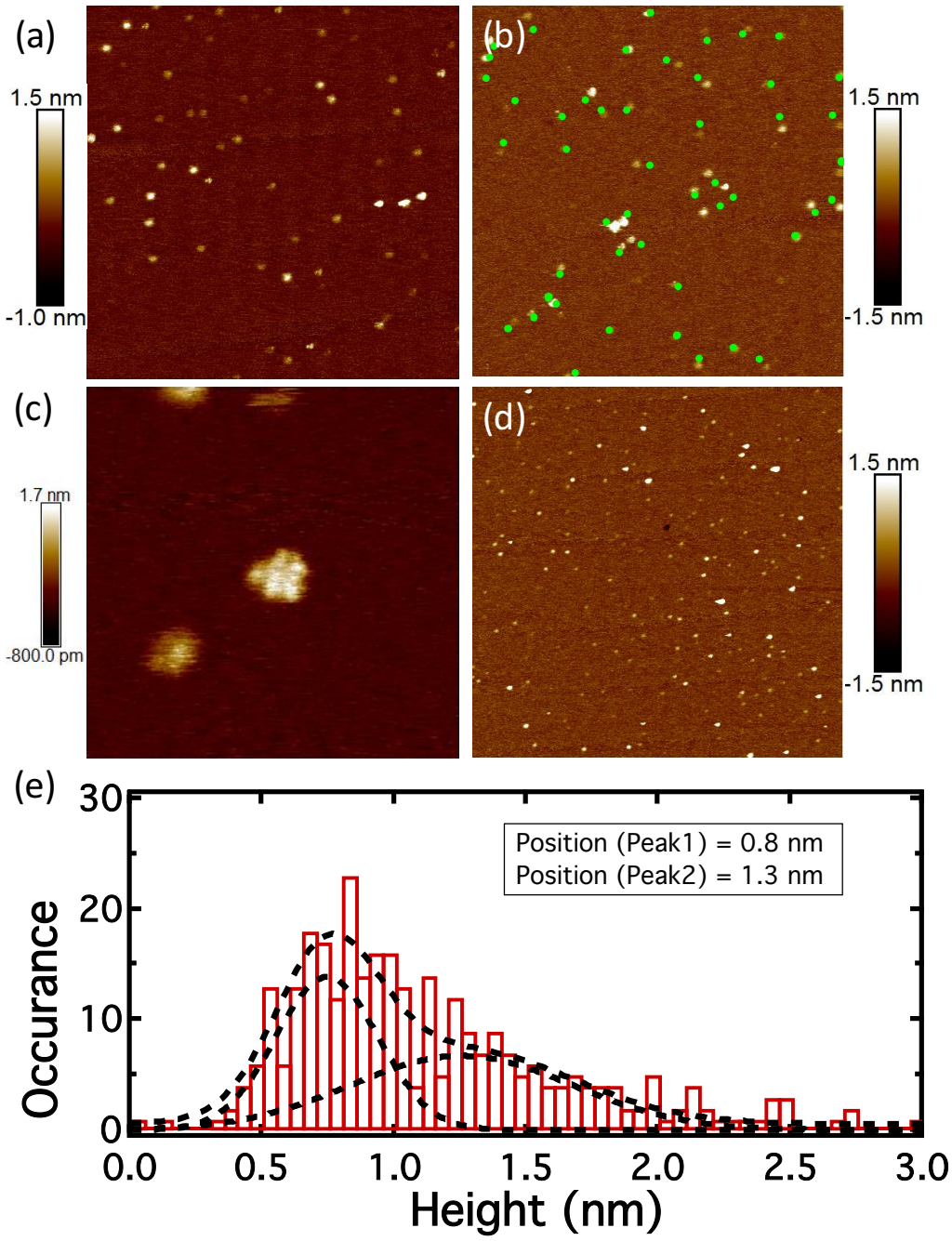


Figure S5. The AFM image of DOPC lipid bilayer on mica as a control experiment for AFM images of pR-containing DOPC lipid bilayer on mica. (a) height image of 1 μm scan; (b) line profile along the dashed line in (a).

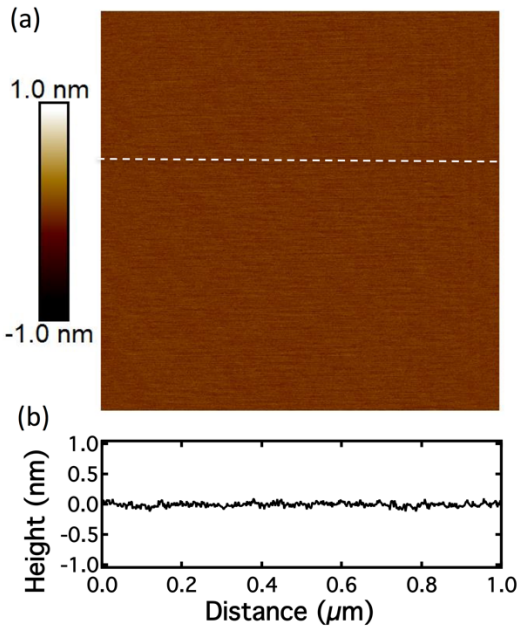


Figure S6. The TIRF image of pR-Alexa488 molecules in DOPC lipid bilayer on mica as a control experiment, which showed almost all of the pRs were immobile in the lipid bilayer. (a) TIRF image at time = 0 s; (b) TIRF image at time = 5s.

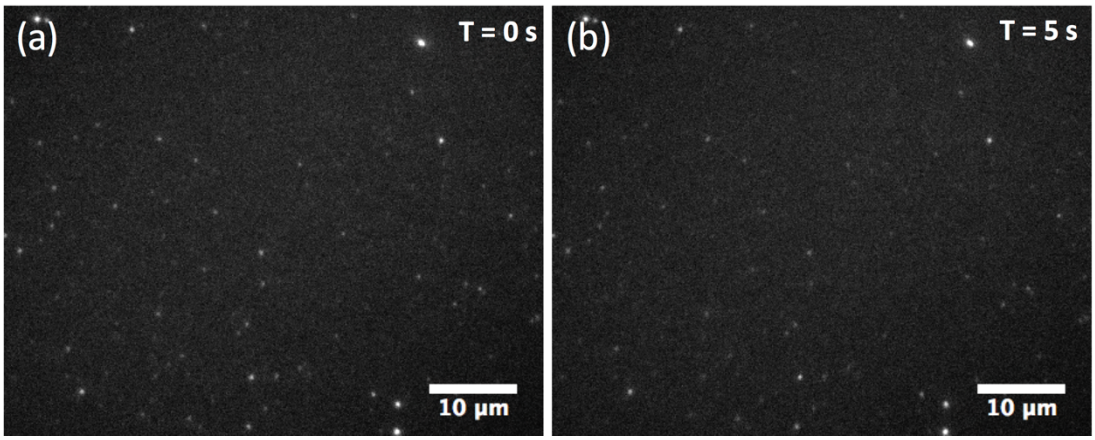
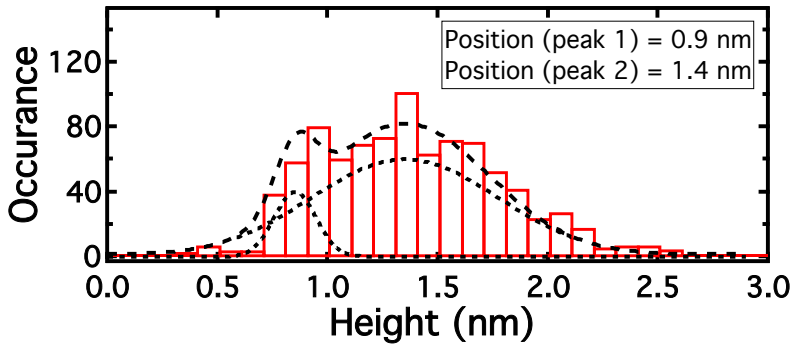


Figure S7. The histogram and two-peaks fitting of the center heights of pRs in lipid bilayer on glass after application of electric field. Peak 1: position = 0.9 nm; FWHM = 0.2 nm; area% = 11%. Peak 2: position = 1.4 nm; FWHM = 0.9 nm; area% = 89%.



Experimental Details:

Purification of pRs and labeling of pRs

The plasmid containing the *pR* gene (pBAD-pR) was transformed into BL21 competent cells. [1] Single colonies of *E. coli* strains were inoculated into 6 ml of LB medium (plus ampicillin) and grown overnight at 37 ºC in the dark with shaking. Next day x/66 ml of the overnight culture was used to inoculate x ml of LB medium (plus ampicillin) in a conical flask and allowed to grow for 2 hrs at 37ºC with shaking. Cells were induced with a final L-arabinose concentration of 0.02 % and 5 mg/ml for all-trans retinal. Cells were allowed to grow for a further 4 hrs before being spun down and frozen at -20 ºC until further use. The pR protein was purified using the same protocol use by Gourdon *et al*. [1]

The pR protein (1mg/ml) in 20mM NaH_2PO_4 150mM NaCl 1% octyl-β-D-glucopyranoside (β-OG) pH 7.4 was mixed with 10x excess of Alexa Fluor® 488 C_5-maleimide (Thermo Fisher Scientific). The mixture was left in the dark for 2 hrs. 1mM DTT was added to the mixture for 20min before passing it through a desalting column equilibrated with 10mM Mops pH7.4 20mM $MgCl_2$ 1% (β-OG).

Reconstitution of pRs

The reconstitution of pR was undertaken using the Bio-beads method. [2] In detail, 1mg 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC) lipids (Avanti Lipids, Inc. USA) in chloroform/methanol (1:1) solution were dried under nitrogen flow for 30min, which was then dissolved in 1ml β-OG buffer solution (300 mM NaCl, 40mM MOPS, pH7.4, 1 w% β-OG). 100 ml pR-Alexa488 with a concentration of 0.1 mg/ml was added to above lipid-detergent solution and kept at 4 ºC for 1h. Afterwards, 4 batches of 30 mg pretreated bio-beads were added to the protein-lipid solution at

every 3 hours. [2] At last, the proteoliposome and bio-beads were separated by centrifugation at 3000 rpm for 2 min.

Patterned lipid bilayer formation

Small unilamellar vesicles for bilayer formation were prepared by extruding the proteoliposome solution, obtained above, through a polycarbonate membrane (with 50 nm pore size) for 11 times using a liposome extrusion kit (Avanti Lipids, Inc. USA). [3] For bilayer formation, a glass substrate (22 mm x 26 mm x 0.15 mm) (Menzel Co. Germany) was cleaned in acetone for 5min, Milli-Q water for 5 min, and then piranha solution (H_2SO_4 : H_2O_2 = 7:3) for 20 min. Afterward, it was rinsed with milli-Q water and then dried under nitrogen flow. Immediately prior to use, the slides were cleaned again with 20 min UV-ozone cleaning (UVOCS Inc., USA).

For patterned lipid bilayers, a thin layer of fibronectin (~ 10 nm by AFM) was patterned on the glass slide by microcontact printing using a PDMS stamp. [4] The fibronectin pattern was the negative of the bilayer pattern to be formed.

The glass slide was fixed into a home built flow-cell, which was then filled with 0.2 mg/ml proteoliposome and incubated for 30 min at room temperature. [5] The flow-cell was rinsed with Milli-Q water, flow rate 0.75 ul/min, for one hour to remove the remaining proteoliposome from the flow-cell.

FRAP experiment

An epifluorescence microscope (E600 Nikon, USA) was used for fluorescence recovery after photobleaching (FRAP) experiments. A high-pressure mercury arc lamp was used for the lighting and bleaching of fluorescence samples. The bleaching spot was 28 μm in diameter and bleaching time was fixed at 30 s. A series of time-

lapse fluorescence images after photobleaching was collected using a Zyla sCMOS CCD camera (Andor Technology Ltd, Belfast, UK). The diffusion coefficient and mobile fraction of pR and D291 was calculated using Axelrod method. [7]

Single molecule tracking (SPT)/ Total internal reflection fluorescence

A home-built total internal reflection fluorescence microscope (TIRFM) was used for the tracking the movement of single molecule of pR-Alexa488 in lipid bilayer on mica.[8] The series fluorescence images were obtained using a x60 objective, at 7 frames/s using a 491 nm laser source with a maximum of 50 mW output.

Atomic force microscopy (AFM) experiments

The AFM images of pR molecules in the DOPC lipid bilayer on mica or glass (i.e. reference samples) were obtained by using a FastScan atom force microscope (Bruker, USA), operated in tapping mode, using Fastscan-DX cantilevers (Bruker) with a nominal spring constant of 0.25 N m^{-1} and a nominal tip radius of 5 nm. The scan rate was 5 - 10 Hz.

The AFM images of pR molecules in the DOPC lipid bilayer on glass (after the application of E field) were obtained by using an Asylum Research MFP-3D atom force microscope (Asylum, UK), operated in intermittent contact mode, using SNL cantilevers (Bruker) with a nominal spring constant of 0.24 N m^{-1} and a nominal tip radius of 2 nm. The free oscillation amplitudes were ca. 10 nm and the amplitude setpoint was adjusted to maintain imaging forces between 140 - 320 pN. The scan rate was 2 Hz, corresponding to tip scan speeds of 1 - 4 μm s^{-1}.

Electrophoresis of pR in lipid bilayer

Electrophoresis experiments were carried out in a home-built flow cell, which had two platinum electrodes (3 cm apart). [5b] A DC voltage (200 V) was applied between the two electrodes to create an electric field of 66.7 V/cm. A constant flow of degassed Milli-Q water (0.75 mL/min) was used throughout the experiment to remove any gas bubbles generated at the electrodes. The movement and accumulation of pR-Alexa488 and D291 in the lipid bilayer was monitored by epifluorescence microscopy (E600 Nikon, USA). The analysis of the built-up of the fluorescent molecules in the trap head regions is following the method described previously. [5b]

References

[1] P. Gourdon, A. Alfredsson, A. Pedersen, E. Malmerberg, M. Nyblom, M. Widell, R. Berntsson, J. Pinhassi, M. Braiman, Ö. Hansson, N. Bonander, G. Karlsson, R. Neutze, *Protein Expression and Purification* **2008**, *58*, 103-113.

[2] P. W. Holloway, *Analytical Biochemistry* **1973**, *53*, 304-308.

[3] M. J. Hope, M. B. Bally, G. Webb, P. R. Cullis, *Biochimica et Biophysica Acta (BBA) - Biomembranes* **1985**, *812*, 55-65.

[4] aL. A. Kung, L. Kam, J. S. Hovis, S. G. Boxer, *Society* **2000**, 6773-6776; bA. Bernard, J. P. Renault, B. Michel, H. R. Bosshard, E. Delamarche, *Advanced Materials* **2000**, *12*, 1067-1070.

[5] aP. Bao, M. R. Cheetham, J. S. Roth, A. C. Blakeston, R. J. Bushby, S. D. Evans, *Analytical Chemistry* **2012**, *84*, 10702-10707; bM. R. Cheetham, J. P. Bramble, D. G. G. McMillan, L. Krzeminski, X. Han, B. R. G. Johnson, R. J. Bushby, P. D. Olmsted, L. J. C. Jeuken, S. J. Marritt, J. N. Butt, S. D. Evans, *Journal of the American Chemical Society* **2011**, *133*, 6521-6524.

[6] M. J. Ranaghan, S. Shima, L. Ramos, D. S. Poulin, G. Whited, S. Rajasekaran, J. A. Stuart, A. D. Albert, R. R. Birge, *The Journal of Physical Chemistry B* **2010**, *114*, 14064-14070.

[7] D. Axelrod, D. E. Koppel, J. Schlessinger, E. Elson, W. W. Webb, *Biophysical Journal* **1976**, *16*, 1055-1069.

[8] A. Kusumi, T. A. Tsunoyama, K. M. Hirosawa, R. S. Kasai, T. K. Fujiwara, *Nat Chem Biol* **2014**, *10*, 524-532.