SUPPORTING INFORMATION

Site-specific immobilization and micrometer and nanometer scale photopatterning of yellow fluorescent protein on glass surfaces

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TITLE RUNNING HEAD Patterned light harvesting complexes

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EXPERIMENTAL DETAILS

Purification of the Histidine Tagged- YFP

The gene sequence of YFP was amplified by PCR from pCS2-Venus vector (a kind gift from Dr. Atsushi Miyawaki, RIKEN Brain science institute, Japan). The resulting *Nde I /Bam HI* fragment was cloned into a pET9a (Novagen) expression vector which had been modified to contain the MCS and

Histidine-tag sequence from pET14b (Novagen). YFP-His_6 protein was produced by heterologous expression in *E. coli* (BL21); cells were grown to an $O.D_{680}$ of 0.6 at 25 °C then induced using IPTG (0.4 mM) for 12 hrs at 20 °C. Pelleted cells (2000 x g / 10 min) were lysed by sonication and the resulting lysate was clarified by a further spin (5000 x g / 20 min). YFP was purified to homogeneity from clarified lysate using a ProPur^{TM} Ni-NTA spin column (Nunc) as detailed in the manufacturer's instructions. Protein was verified to be fluorescently active by monitoring excitation at 500 nm and emission at 535 nm. Presence of the His_6 tag on the protein was confirmed by western blotting using anti-His_6 antibodies (Novagen).

Preparation of Monolayers on Oxide Substrates

SAMs of (3-mercaptopropyl)trimethoxysilane (MPTMS, Sigma Chemical Company, Poole, UK) were prepared on either glass microscope slides (Chance Proper no. 2 thickness, size 22 mm × 64 mm) or, prior to XPS analysis, silicon wafers (Agar Scientific, Stansted, UK) which were cleaned using "Piranha solution" (70 % sulfuric acid (>95%, Fisher Scientific, Loughborough, UK): 30% hydrogen peroxide, 100 vol. (Sigma Chemical Company)) and then treated with RCA solution (Radio Cooperative of America, 20% hydrogen peroxide, 100 vol., 20% Ammonium Hydroxide (>95%) (Sigma Chemical Company) and 60% de-ionized water (18.2 MΩ)) for 1 h at 80^{o}C in order to create a homogenous hydroxyl surface. The surfaces were rinsed in a copious amount of de-ionized water and dried. The cleaned substrates were immersed in a 5 mM solution of (3-mercaptopropyl)trimethoxysilane in anhydrous tetrahydrofuran (THF, >99%, Sigma Chemical Company) for 90 min in an anhydrous, dark and inert nitrogen atmosphere. The SAMs were then rinsed in THF and HPLC grade ethanol (Sigma Chemical Company) and sonicated for 15 min in THF in order to remove any aggregates that may have formed on the surface. Finally the SAMs were placed in a Vacuum oven at 120 ^{o}C (Fistreem International, Loughborough, UK), for 1 h in order to remove any solvent remaining on the surface. Exposure of the materials to light was minimized at all times to prevent unwanted oxidation of the thiol groups on the siloxanes. The receding contact angle of all the SAMs was determined using a contact

angle goniometer (Ramé-Hart Instrument co., Netcong, USA) and found to be 63 $\pm 5^{o}$.

Immobilization of OEG to Thiol Terminated Monolayers

Surfaces to be used in the OEG degradation experiments were then immersed in a 2 mM solution of methyl-PEO12-maleimide (Perbio Science, Cramlington, UK) in de-ionized water (18.2 MΩ) for 12 hours, and then rinsed and stored in de-ionized water. All surfaces were checked by monitoring their receding contact angle, and was found to be 27 ± 8^{o}.

X-Ray Photoelectron Spectroscopy

All XPS was performed using an Axis Ultra DLD X-ray photoelectron spectrometer (Kratos Analytical Ltd., Manchester, UK). All S2p scans were performed at a pass energy of 80 eV, all C1s scans were performed at a pass energy of 20 eV. Samples were kept under a vacuum of 10^{-8} mbar for approximately 1 h prior to analysis. Resulting XPS spectra were analyzed using the CasaXPS program (Casa, http://www.casaxps.com, UK). All curve resolution was conducted using a linear baseline. Peaks were fitted using Gaussian curves. The full width at half maximum counts (FWHM) of all components was constrained equal to that of the aliphatic carbon or the $S2p_{3/2}$ component of un-oxidized sulfur depending on the scan, but all other parameters were left free to vary during fitting.

Lithography

Photolithography was conducted using a frequency doubled argon ion laser emitting light with a wavelength of 244 nm (Coherent FreD 300C, Coherent, Ely, UK). For micron-scale patterning 1500, 1000 and 600 mesh electron microscope grids (Agar Scientific, Stansted, UK), consisting of 16.9 μm^2 x 16.9 μm^2, 25.4 μm^2 x 25.4 μm^2 and 42 μm^2 x 42 μm^2 square openings, respectively, were used as masks.

Photochemical Degradation of OEG derivatized SAMs

The OEG surfaces were irradiated at a power of 50 mW. The power incident on the surface was ca. 68% of the laser power and the illuminated area was 0.2 - 0.4 cm^2 leading to an incident power of 85 - 170 mW cm^{-2} and a net exposure at the surface in the range of 5.1 J cm^{-2} - 10.2 J cm^{-2} for a 1 min

exposure. For SNP, the laser was coupled to a ThermoMicroscopes Aurora III near-field scanning optical microscope (Veeco Instruments Ltd., Cambridge, UK) fitted with a Al coated HF etched fiber probe with an aperture of around 150 nm (Jasco UK, Great Dunmow, Essex). During near-field lithography, the laser power coupled to the fiber was 3 mW, and the probe velocity was 0.3 μm s^{-1} during the fabrication of the lines.

Protein Immobilization of Photo-chemically degraded OEG surfaces

The patterned OEG derivatized monolayer was immersed in a 5 mM solution of N-(5-amino-1-carboxypentyl)iminoacetic acid (AB-NTA, NBS Biologicals) in de-ionized water for 1-2 h and rinsed in de-ionized water. An alkylimino-de-oxo-bisubstution reaction occurs between the primary amine on the NTA and the aldehyde degradation product of the OEG, causing the NTA to be bound to the surface via an imine bond. The surface was charged with nickel atoms and the histidine tagged YFP was bound as in the above procedure.

Atomic Force Microscopy

Tapping mode AFM images were acquired on a Nanoscope Multimode IV atomic force microscope (Veeco Instruments Ltd.) with all measurements performed in air. The probes used were silicon nitride nanoprobes (Veeco Instruments Ltd.). The nominal force constant of these probes was 50 N m^{-1}.

Confocal Microscopy

Patterned histidine tagged YFP surfaces were imaged using a LSM 510 meta laser scanning confocal microscope (Carl Zeiss, Welwyn Garden City, UK). The patterned surfaces were placed in well plates and immersed in de-ionized water. A 63 x magnification water dipping lens was used for imaging. The 514 nm band of an Ar laser (1.5 mW) was used to excite the YFP and the fluorescence was collected at wavelengths above 535 nm. A band pass filter operating between 535 – 590 nm was used to block the incident light from the detector.

Confocal Microscopy image at high resolution

To check whether the patches observed in some AFM images were composed of non-specifically

adsorbed proteins or other material deposited from the buffer, high magnification images were formed of micropatterned samples. Figure 1(a) shows a representative image. It is plain that there is no fluorescence from the bars (masked regions) indicating that the patches observed there were not composed of protein.

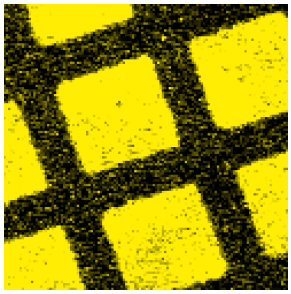
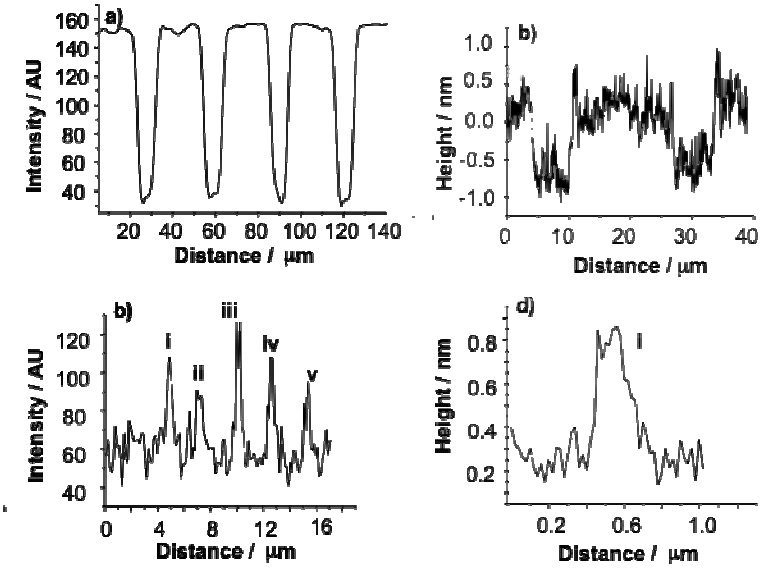


Figure 1. Confocal image showing a region of the sample shown in figure 3(a) in the main text at higher magnification.

Line section data from confocal and AFM investigation of patterns

Figure 2 shows line sections through the fluorescence images of the micropatterned sample shown in figure 3(a) and the nanopatterned sample in figure 3(c) in the main text together with cross sections through the AFM images of the micro- and nano-patterned samples. The cross-section through the fluorescence image of the micropatterned sample (figure 2(a)) exhibits a regular shape, and the regions of low intensity, where the OEG surface remains intact, are uniform, indicating that there is little non-specific absorption of YFP to these regions. All of the exposed regions exhibit uniform contrast too, indicating a homogenous coating of protein at the surface. There is a low background fluorescence signal. The cross-section through the confocal microscope image of the YFP nanolines (figure 2(c)) shows the widths of 3 lines drawn by SNP (labeled (i), (iii) and (v)) and also two diagonal lines (labeled (ii) and (iv) which arise from the fact the light source is not blocked off when the probe moves diagonally between lines during the lithographic process. The diagonal lines arise from a single sweep of the probe and thus represent the point at which the resolution is best; the other lines result from two sweeps of the probe along the same line and because of hysteresis in the scan stage of the SNOM used

here, they do not exactly overlap, leading to an increased line width. The 5 lines shown in figure 2(c) have an average FWHM of 393 nm, but on the diagonals (single-sweep), a FWHM of 262 nm was measured, which is effectively the diffraction limit for this microscope using illumination at 514 nm. Figure 2(d) is a line section taken from a tapping mode AFM image of the nanopatterned sample. It crosses one of the diagonal lines of YFP where hysteresis in the SNOM sample stage is not a problem.



Here the FWHM is shown to be 179 nm, close to the diameter of the aperture in the probe used (150 nm). The height of the line is ca. 0.8 nm, indicating the formation of a monolayer of site specifically bound protein.

Figure 2. Cross-sections through micrographs in figure 3(a, c, d) in main text. (a) Cross-section through confocal image of micron scale patterns of histidine tagged YFP: (b) cross-section through tapping mode AFM image of micropatterned sample; (c) cross-section through confocal images of nanolines of histidine tagged YFP (average FWHM = 393 nm); and (d) cross section through tapping mode AFM image of nanolines of histidine tagged YFP (FWHM = 179 nm). The regions that the image cross-sections are taken from are indicated by the arrows in figure 3 in the main text.