Manufacturing of Anisotropic Particles by Site Specific Oxidation of Thiols

Kristofer Eriksson, LarsErik Johansson, Emmanuelle Göthelid, Leif Nyholm and Sven Oscarsson

Supporting Information

Experimental.

Chemicals and substrates: Unless otherwise noted, all chemicals were obtained from commercial suppliers and used without further purification. Magnetic latex polymer particles with amino functionality (Micromer® –M PEG-NH_2) were obtained from Micromod Partikeltechnologie GmbH and PD-10 gel-filtration columns were from GE Healthcare. The silicon wafers (Si (001)) and the glassy carbon working electrode were purchased from Goodfellow.

Thiol-functionalisation of supports: Magnetic latex polymer particles, Micromer® –M PEG-NH_2, (100 µL, 7×10^8 particles/mL) were washed three times in 1mL PBS (10 mM phosphate, 150 mM NaCl, 10 mM EDTA, pH 7.4) and resuspended in 1000 µL PBS. N - Succinimidyl 3-(2-pyridyldithio)-propionate (SPDP) (25 µL, 20 mM in DMSO) was added to the bead suspension and reacted for 90 minutes. The beads were washed five times with 1000 µL PBS, resuspended in 1000 µL PBS and kept at 4 ºC. The Micromer –M PEG-SS-Pyridyl particles in 1000 µl suspension (7×10^7 particles/mL) were collected with an external magnet and the buffer was changed to 1000 µL dithiothreitol (DTT) in acetate buffer (pH 4.5, 2 % w/v DTT) and incubated at room temperature for 20 min. The beads were washed five times with PBS (10 mM phosphate, 150 mM NaCl, pH 7.4), resuspended in 80 mL PBS to a final concentration of 8.75×10^6 particles/mL and kept at room temperature.

The amount of thiol groups on the particles was determined spectrophotometrically by reacting the thiolated particles with 2, 2´-dipyridyl-disulphide (2PDS) as previously described

[1]. It was found that approximately 1.0×10^8 thiol groups were attached to each bead. The thiolated particles were also conjugated with fluorescent Immunoglobulin G (IgG) and investigated using fluorescence microscopy. Since the reacted particles exhibited homogeneous fluorescence it was concluded that the thiol groups were evenly distributed on the surfaces of the particles.

A silicon surface (0.8x0.8cm) was silanised with (3 -mercaptopropyl) methyl dimethoxy silane (MPMDMS). Prior to silanisation, the silicon wafer was cleaned by piranha etch (i.e. 2/3 sulphuric acid, 1/3 (30%) hydrogen peroxide) for 10 minutes followed by extensive washing with distilled water. The procedure was repeated twice. After the last washing step with water, the silicon wafer was blown dry with a flow of nitrogen gas. Then the wafer was put into a beaker with MPMDMS (2%, v/v) in toluene. After 2 hours reaction the Si wafer was washed by ultrasonication in toluene followed in distilled water during 10 minutes each. Finally the thiol-functionalised silicon wafer was blown dry with a flow of nitrogen gas.

Potentiostatic experiments: Thiolated beads (2 mL in PBS (10 mM phosphate, 150 mM NaCl, 10 mM EDTA, pH 7.4, unless stated otherwise), 8.75×10^6 beads/mL) were added to the reaction cell and allowed to distribute on the working electrode surface by the forces of gravity and magnetic field for 2 min. The experiments with the gold electrode were carried out analogously employing a pH 7.4 PBS solution lacking EDTA and with a KCl concentration of about 2 mM. Potentials spanning from +1.3 to +2.31 V versus Ag/AgCl were applied for 0.1 to 60 seconds. In the experiments involving oxidation of particles with the gold electrode, an Ag/AgCl reference electrode was used while a platinum quasi reference electrode was employed in all other experiments. As the potential of the platinum quasi reference electrode was found to be +0.31 V vs. the Ag/AgCl electrode, this value was used to convert the Pt quasi reference potentials to the Ag/AgCl scale. The beads were thereafter resuspended and the volume reduced to 50 μL by collecting the beads with a permanent

magnet. Between the different oxidations of thiolated beads the cell as well as the working, counter and reference electrodes were washed with PBS.

A thiolated silicon surface was typically placed directly on to the glassy carbon working electrode with its polished side facing the electrode in a 3 ml solution of PBS (10 mM phosphate, 150 mM NaCl, 10 mM EDTA, pH 7.4). A potential of +2.31 V vs. Ag/AgCl was applied during 60 seconds. The oxidised silicon surfaces were then kept in a solution of acetate buffer pH 4.5 prior to the X-ray photoelectron spectroscopy measurements.

X-ray Photoelectron Spectroscopy (XPS): The electrochemically oxidised silicon wafers were investigated with XPS employing a Scienta ESCA-300 spectrometer using monochromatic Al-K_α radiation with photon energy of 1487 eV. Overview, Si2p, S2p and S2s photoelectron spectra were recorded, although only the latter is shown in this communication. All binding energies were referred to the Si2p core level at 99.3 eV. The spectra were recorded at normal emission of the photoelectrons (90 degree take-off angle).

FITC labelling and thiolation of IgG: IgG (2 mg) (Human) was dissolved in 1 mL carbonate buffer (0.1 M $NaHCO_3$, pH 9.0). Fluorescein isothiocyanate (FITC) (200 µL, 1 mg/ml in carbonate buffer), was then added to the protein solution. After 24 hours reaction at 4°C in the dark, the buffer was changed to PBS by gel-filtration (PD-10). IgG-(FITC) (1000 µL, 2 mg/mL) in PBS was mixed with N -Succinimidyl 3-(2-pyridyldithio)-propionate (SPDP) (25 µL, 20 mM in DMSO). After 24 hours reaction at 4°C, the buffer was changed to PBS by gel-filtration. IgG-(FITC) SS-Pyridyl (1000 µL, 2 mg/mL) was mixed with 50 µL 100 mM dithiothreitol in PBS. After 20 min reaction, the buffer was changed to PBS by gel-filtration.

IgG functionalisation of partial oxidised beads: Bead suspension from the electrochemical oxidation was immediately mixed with 50 µL freshly prepared IgG-(FITC)

SH (2 mg/mL in PBS) and incubated for 1 hour and was thereafter washed 3 times with 200 μL PBS.

Fluorescence microscopy: The bead surfaces were studied in a Nikon Eclipse fluorescence microscope equipped with a Nikon Coolpix camera. The fluorescence filter was tuned to provide an excitation wavelength of 494 nm and an emission wavelength of 520 nm.

Size determination of fluorescent spots: The images of the beads with the fluorescent spots were evaluated with the image processing and analysing programmed ImageJ which is freely available on Internet (http://rsbweb.nih.gov/ij/).

Generation and characterisation of thiol supported Au(I) species.

General: When thiolated beads were electrochemically oxidised using a gold working electrode both partial- and weak homogenous oxidation of the whole bead surfaces could be obtained. This phenomenon was investigated with X-ray Photoelectron Spectroscopy (XPS) employing an electrochemically oxidised thiolated silicon surface as is described below.

Experimental: A thiolated silicon surface was typically placed directly on top of a gold working electrode in a 3 ml solution of PBS (10 mM phosphate, 150 mM NaCl, 10 mM EDTA, pH 7.4). A potential of +1.21 V vs. Ag/AgCl was applied for 10 seconds. The silicon surface was then washed in PBS solution followed by rinsing in water and was then blown dry with nitrogen. The presence of Au species on the silicon surface was detected by studying the Au4f region with XPS employing a Scienta ESCA-300 spectrometer using monochromatic Al-K_α radiation with photon energy of 1487 eV. All binding energies were referred to the Si2p core level at 99.3 eV and the spectra were recorded at normal emission of the photoelectrons (90 degree take-off angle). Spectra were also recorded for a clean gold surface for comparison. The $Au4f_{7/2}$ and $Au4f_{5/2}$ doublet for the clean gold surface and the oxidised thiolated silicon wafer, respectively, are displayed in Figure S1. The binding

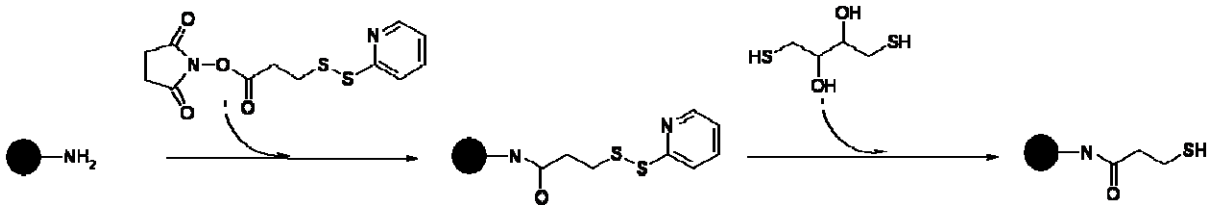
energies (BE´s) for the Au4f$_{7/2}$ signal for the metallic gold (a) and the oxidised thiolated silicon surface (b) can be seen to be 84.0 eV and 85.5 eV, respectively. BE´s around 85.5 eV are typical values found for Au(I) species [2]. No gold species were found on the silicon surface when it was placed on top of the gold working electrode in the absence of an applied potential.

References

[1] K. Brocklehurst, J. Carlsson, M. P. J. Kierstan, E. M. Crook, *Biochem. J.*, 133, **1973**, 573-584.

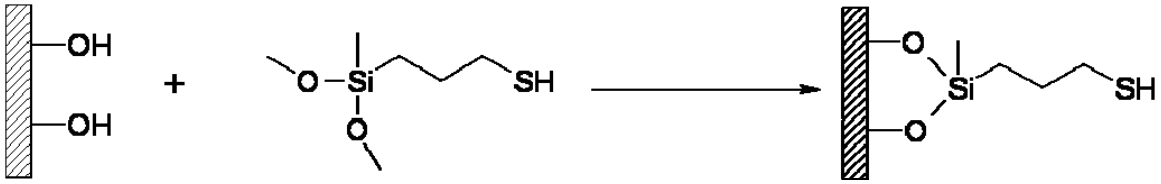
[2] A. McNeillie, D. H. Brown, W. E. Smith, *J. C. S. Dalton*, **1980,** 767-770.

Scheme S1.



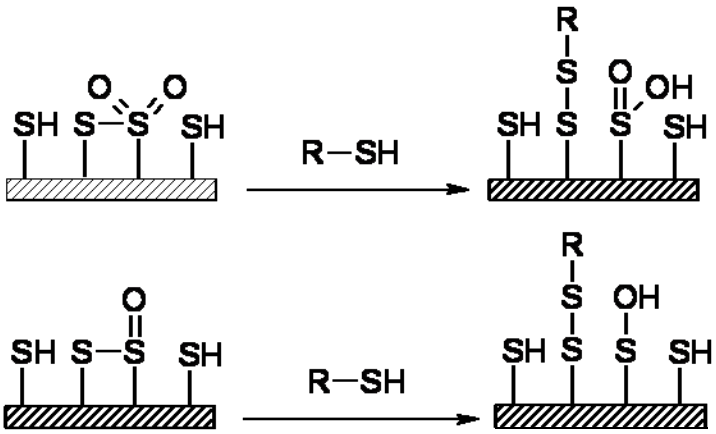
Scheme S1. Thiolation of amino-terminated surfaces e.g. magnetic beads. The surface bound amino groups were reacted with *N* -Succinimidyl 3 - (2-pyridyldithio) - propionate (SPDP) to introduce SS-Pyridyl groups which afterwards were reduced with dithiothreitol (DTT) to yield thiols.

Scheme S2.



Scheme S2. Thiolation of a silicon surface using mercaptopropyl - methyl dimethoxy silane (MPMDMS).

Scheme S3.



Scheme S3. Protein-functionalisation of the electrochemically oxidised thiol-surface. The reactive thiolsulphonates (SO_2) and thiolsulphinates (SO) are forming covalent disulphide bonds with thiolated proteins. R = Protein

Figure S1.

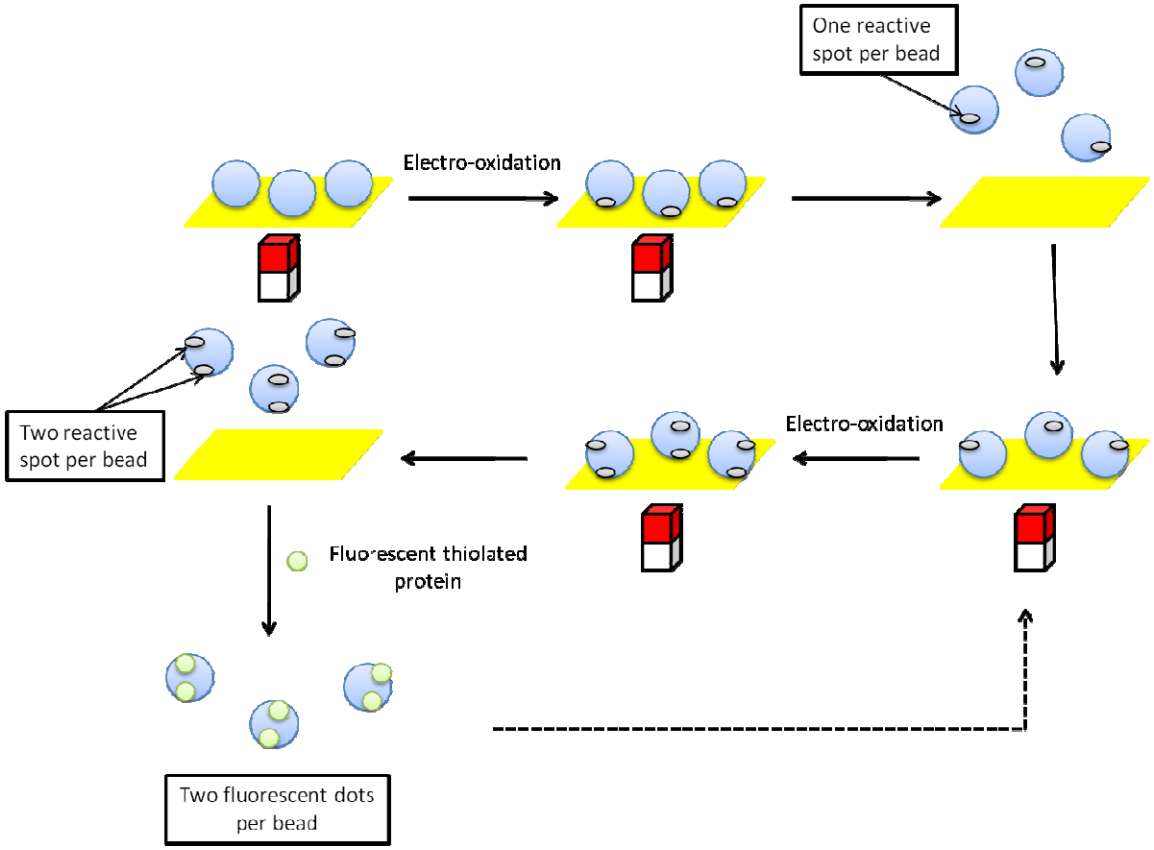


Fig. S1. Formation of patchy-like particles employing several potential steps and subsequent release and attraction of the beads. In this way, new segments of the particle may be oxidised and several areas on the particles can hence be functionalised.

Figure S2.

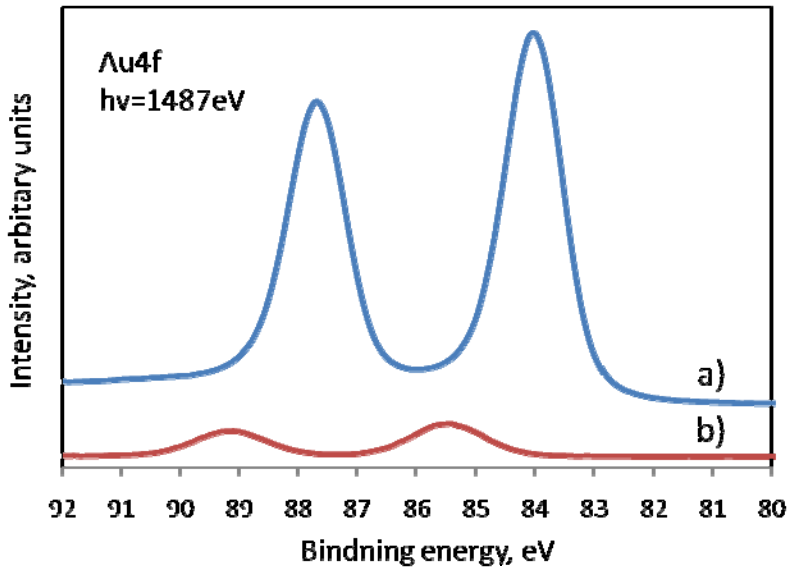


Fig. S2. X-ray photoelectron spectra showing the $Au4f_{7/2}$ and $Au4f_{5/2}$ doublet for (a) a clean gold surface and (b) a thiolated silicon wafer which was electrochemical oxidised on a gold working electrode at a potential of +1.21 V vs. Ag/AgCl during 10 s. The $Au4f_{7/2}$ binding energy at 85.5 eV for (b) is typical for Au(I) species.