Electronic Supplementary Information (ESI)

Protein Immobilisation on Perpendicularly Aligned Gold Tipped Nanorod Assemblies

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Experimental section

Chemicals and Stock Solutions.

Trioctylphosphine oxide (TOPO, 99%), trioctylphosphine (TOP, 90%), cadmium oxide (CdO, 99%) sulfur (S, 99%), Dodecylamine (DDA), (didodecyldimethylammonium bromide (DDAB), Gold Chloride (AuCl), 11-Mercaptoundecanoic acid (99%), 7-Mercapto-1-heptanol (99%) and cytochrome c (cyt c) (horse heart type VI) were all purchased from Sigma Aldrich and used as received. N-octyldecylphosphonic acid (ODPA) was purchased from PCI synthesis. All solvents were purchased from Sigma Aldrich. A stock solution of S in TOP was prepared by weighing out 0.64g of S in 7.64g of TOP in a round bottom flask and left stirring overnight under a flow of argon until S has completely dissolved in TOP.

CdS Synthesis.

Cadmium Sulphide (CdS) nanorods were prepared using standard air free techniques, and redispersed in toluene before use (0.1%w/v).[1] In this method CdS nanorods were synthesized by the injection of a sulfur and tri-*n*-octylphosphine solution into a hot cadmium oxide and surfactant mixture at a high temperature.

Perpendicular Alignment of CdS nanorods.

The nanorods were perpendicularly aligned similar to previous published method; electrophoretic deposition of the nanorods.[2] In this method parallel gold coated copper electrodes were immersed in a dilute solution CdS nanorods in chloroform. The nanorods are positively charged and deposit only on the negative electrode under a

DC electric field of 200 V forming a conformal deposit of perpendicularly aligned nanorods. Deposition occurs on an ITO coated glass slide attached to the negative electrode.

Gold growth on the tips of deposited CdS nanorods.

Gold tips are spun cast onto the nanorods following a previous publication.[3] The gold solution consisted of 22.5 mg of Dodecylamine (DDA), 12.5 mg of didodecyldimethylammoniumbromide (DDAB), 4 mg of AuCl in 2 ml of anhydrous toluene. Typically 5ul of 8.6×10^{-3} mol dm^{-3} of gold solution was spun on the substrate containing the nanorods at 100rpm for 30 seconds before growth was quenched by a few drops of methanol.

Treatment of the surface.

The process of gold nanoparticle growth onto the semiconductor nanorods introduces excess non-coordinating surfactants, namely Dodecylamine (DDA) and Didecyldimethylammonium bromide (DDAB) onto the surface of the previously deposited assemblies. The ligands used in the gold tip growth process are in excess to enable instantaneous reduction of the AuCl onto the nanorods. These excess ligands need to be removed prior to attachment of the thiol linker to the gold nanoparticles. A mild H_2/O_2 plasma is a typical technique to remove excess non-bound surfactant for nanocrystal imaging purposes.[4] The removal of excess ligands is evident in the improvement in image resolution and enhanced contrast without affecting the morphology of the superlattice. The coordinating ligands are necessary for the structural integrity of the nanorod superlattice and their removal would be evident in TEM and SEM analysis by a partial loss of order in the assemblies. Care was taken to ensure this additional cleaning step only removed the extra surfactant deposited in the

gold tipping protocol. Images of the gold tipped nanorod arrays were recorded each time subsequent to this plasma cleaning step.

Alternatively, the nanorods can also be washed in a low concentration of octylamine octylamine for 30 mins prior to thiol attachment. It is important to keep the concentration of octylamine low to avoid rotation and attachment in solution.[5]

Attachment of the thiol.

The electrochemical response of cyt c was examined using 2.5 mM mixed thiol ($HS(CH_2)_{10}COOH$–$HS(CH_2)_7OH$), at a ratio of 1:1, as it has been previously shown to provide faster and more reversible kinetics than a $C_{11}COOH$ SAM. [6] The sample was placed in the mixed thiol solution, solvated in ethanol for ~ 24 hrs.

Immobilisation of cytochrome c.

The gold tipped nanorod sample containing thiols was then immersed in 30 μM cytochrome c solution for a period of 4 hours. The samples were stored in 10 mM phosphate buffer, pH 7.4 until required. The structures were stored in a refrigerator and remained fully functional for several weeks.

Characterization. All samples were rinsed repeatedly before analysis with deionized water to remove any unbound cyt c molecules.

A CHI 630A potentiostat was used for the electrochemical experiments. Sodium chloride (0.1M) was the supporting electrolyte used in electrochemical scans. Deionised water from an Elga-Stat water system was used with a resistivity of 18.2 MΩ. X-Ray Photoelectron Spectroscopy (XPS) were obtained by a Kratos Axis 165 spectrometer using monochromatic Al Kα radiation ($\lambda\nu$ = 1486.58 eV) and fixed

analyser pass energy of 20 eV. All spectra were calibrated using the C 1S at 28418 eV. FT-IR analysis was performed on substrate deposited samples on a Perkin Elemer Spectrum 100 interferometer**.** Scanning Electron Microscopy (SEM) was performed on a Hitachi SU-70 at an acceleration voltage of 3kV**.** Transmission electron microscopy (TEM) was performed using JEOL 2011 TEM with an accelerating voltage of 200kV using a carbon coated copper grid. Fourier Transform Infra Red (FTIR) spectroscopy was performed with a Perkin Elemer Spectrum 100 interferometer.

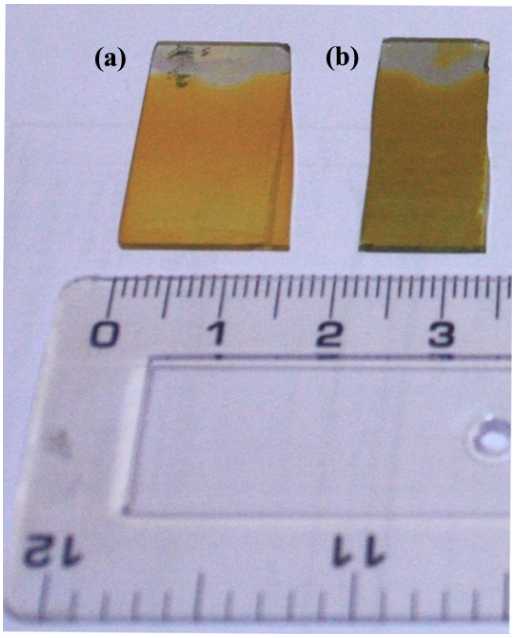

Fig. S1. Photographs of (a) as synthesised nanorod deposition on ITO (b) spin cast gold tips onto previously deposited nanorods

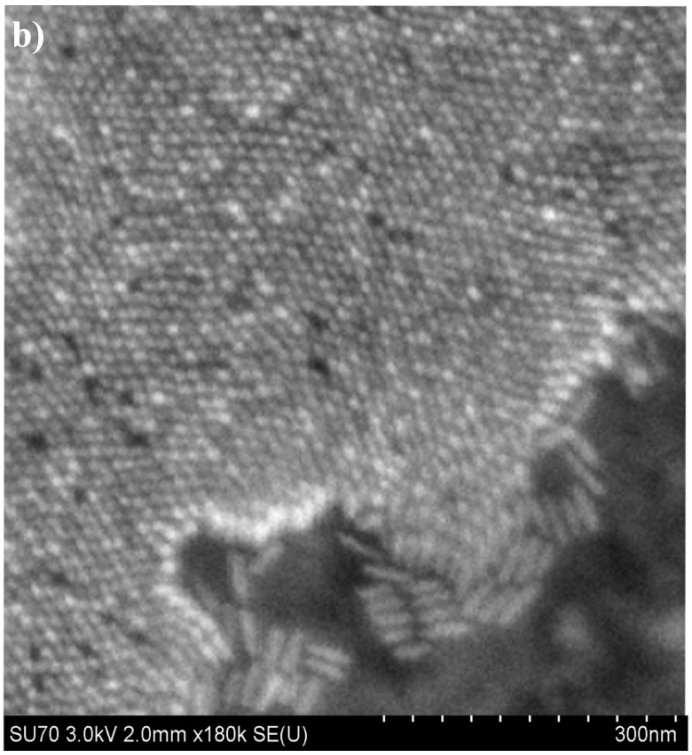

Fig. S2. Top down SEM image showing a perpendicularly aligned nanorods in ITO substrate.

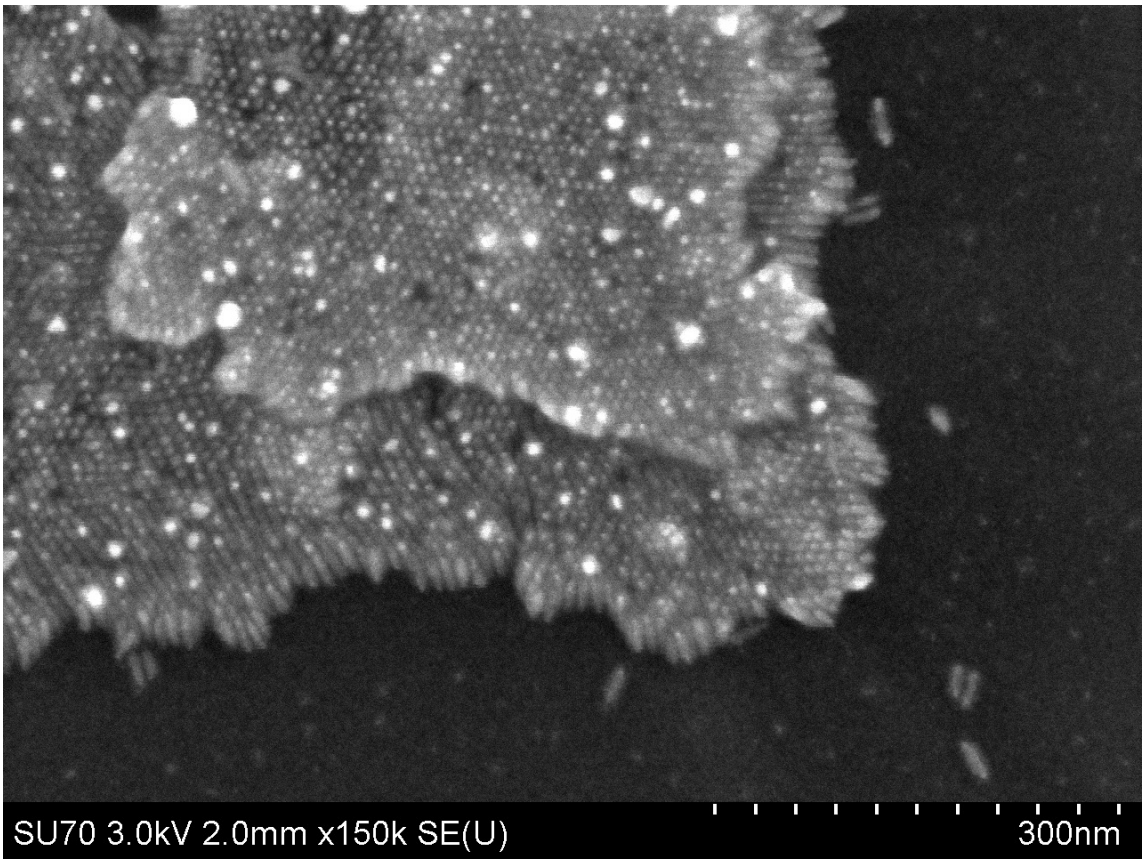

Fig. S3. SEM image showing an edge view of perpendicularly aligned gold tipped nanorods

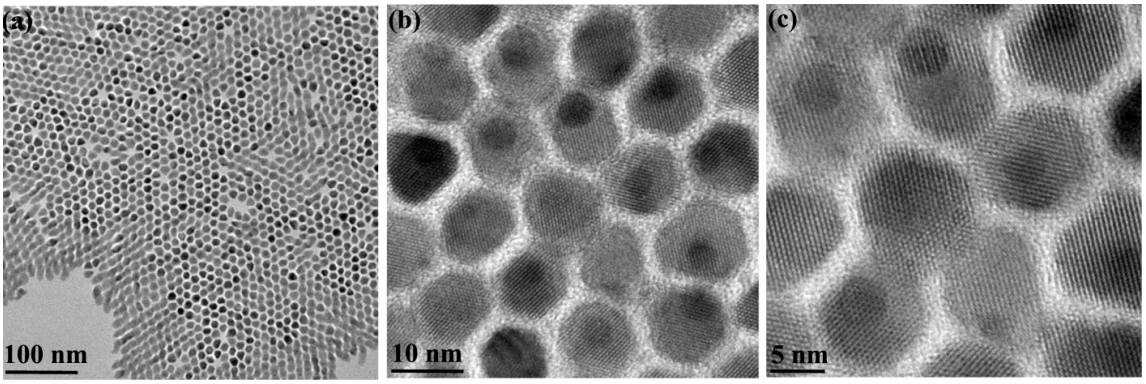

Fig. S4. TEM images at successive magnifications showing 3-4 nm gold tipped perpendicularly aligned nanorod arrays.

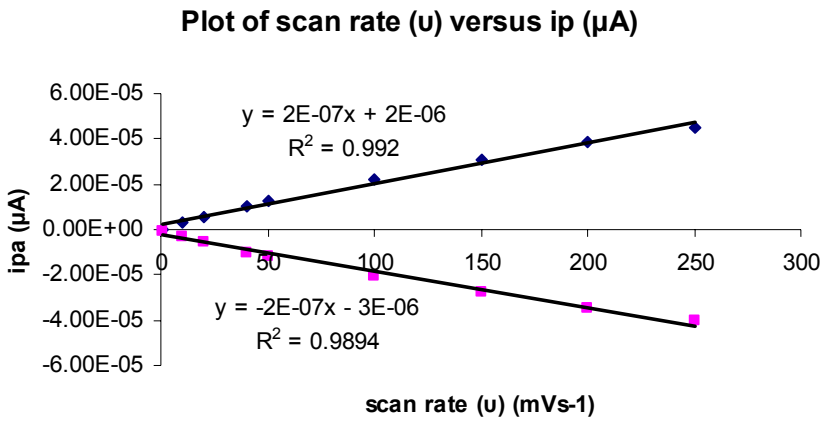

Fig. S5. Linear plots of i_{pa} and i_{pc} vs. scan rate (υ) for cyt c modified electrodes in 10 mM phosphate buffer, pH 7.4.

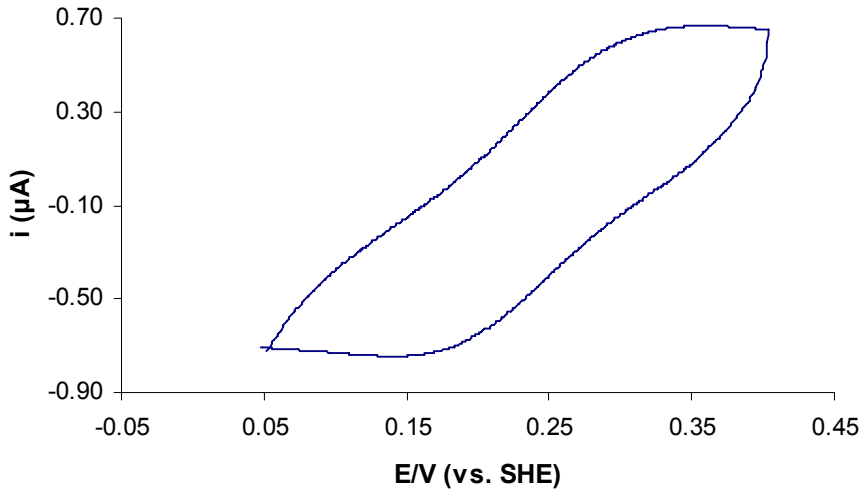

Fig. S6. Cyclic voltammogram (CV) of $C_{10}COOH/C_7OH$ gold tipped nanorods that are parallel to the surface in 10 mM phosphate buffer, pH 7.4 (υ = 20 mVs^{-1}).

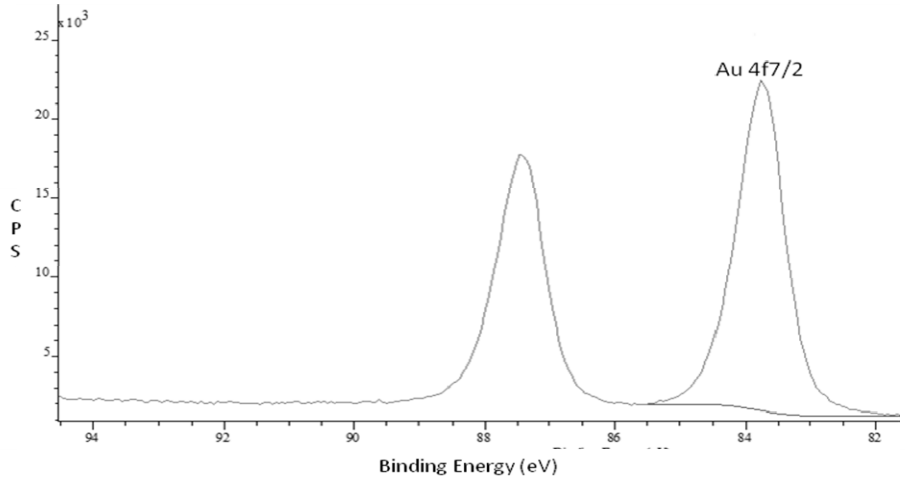

Fig. S7. Au 4f 7/2 photoelectron spectrum from gold tipped nanorods

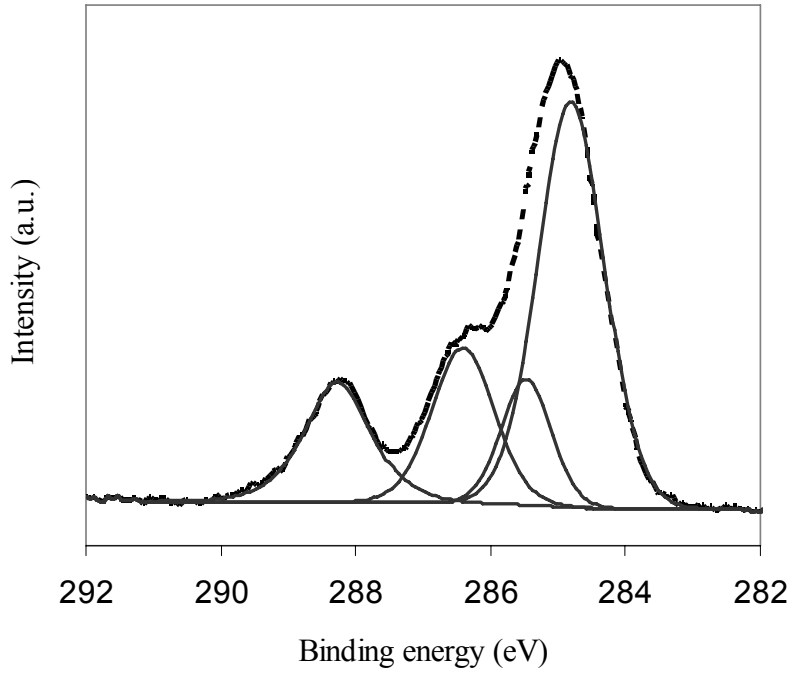

Fig. S8. C 1s photoelectron spectrum from immobilised cyt c on thiol bound gold tipped nanorods highlighting the presence of peptide and carboxylic linkages within the assembly.

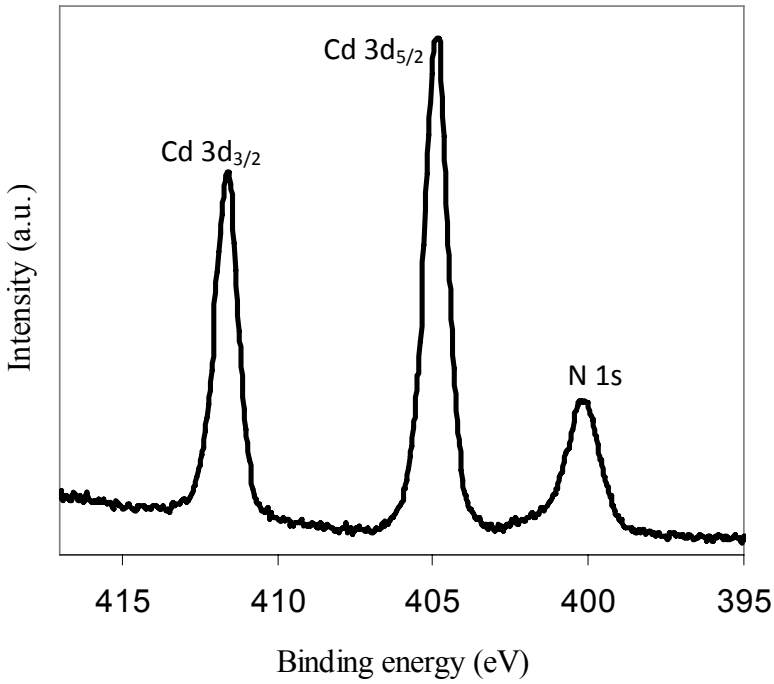

Fig. S9. N 1s photoelectron spectra from immobilised cyt c protein on gold tipped nanorod array. Cd 3d doublet from the nanorod can also be seen.

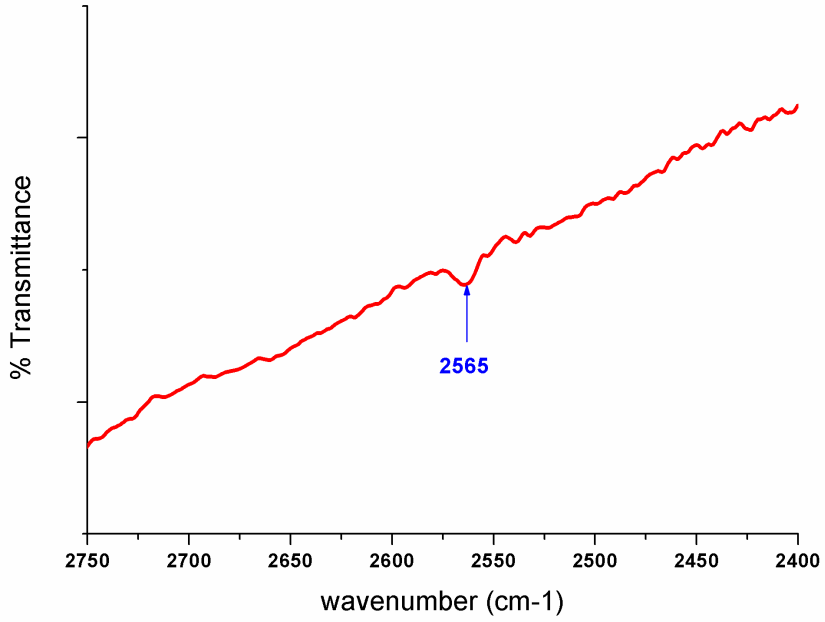

Fig. S10. FT-IR spectra showing the weak S-H stretching absorption peak of the thiol linkers

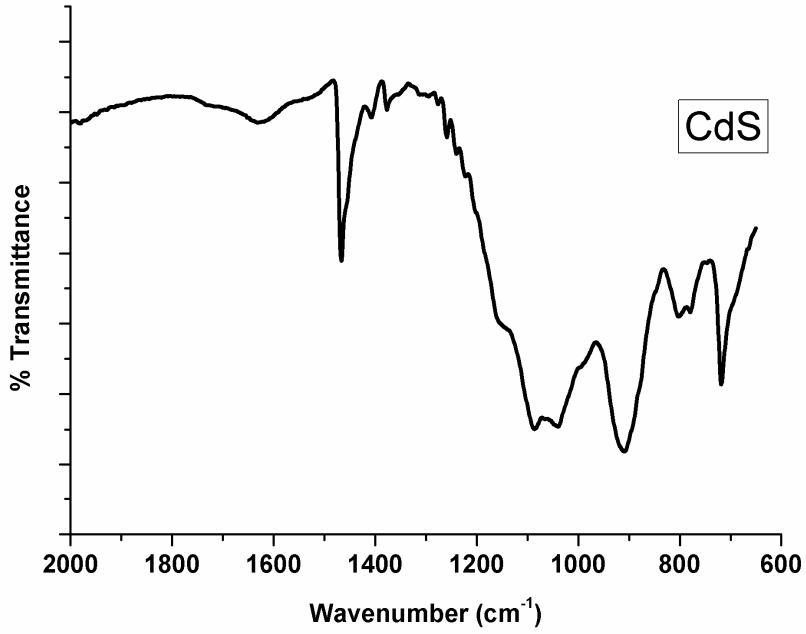

Fig. S11 FT-IR spectra of CdS nanorods

References

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2. S. Ahmed and K. M. Ryan, *Chem. Comm.*, 2009, 6421.
3. O'Sullivan, C., Ahmed S., Ryan, K. M., *J. Mater. Chem.*, 2008, **18**, 5218.
4. McBride, J. R., Lupini, A. R., Pennycook, S. J., Rosenthal, S. J. 'Aberration-Corrected Z-Contrast STEM: Semiconducting Nanocrystals', Dekker Encyclopedia of Nanoscience and Nanotechnology (Second Edition) 2009.
5. O'Sullivan, C., Gunning, R. D., Sanyal, A., Barrett, C. A., Geaney, H., Laffir, F. R., Ahmed, S., Ryan, K. M., *J. Am. Chem. Soc.*, 2009, **131**, 12250.
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