Supporting Information

Materials

Silver shot was purchased from Alfa Aesar (#11357 1-3mm diameter, Premion®, 99.9999%). Tungsten vapor deposition boats were acquired from R.D. Mathis (Long Beach, CA). Polystyrene nanospheres with diameters of 280 ± 4 nm, 390 nm ± 19.5 nm, were received as a suspension in water (Interfacial Dynamics Corporation, Portland or Duke Scientific, Palo Alto, CA) and were used without further treatment. Fisherbrand No. 2 glass coverslips with 18 mm diameters and the buffer salts ($KH_2PO_4.3H_2O$ and KH_2PO_4) were obtained from Fisher Scientific (Pittsburgh, PA). (1*R*)-camphor and 11-mercaptoundecanoic acid (11-MUA) were purchased from Sigma-Aldrich and used as received. For all steps of substrate preparation, water purified with cartridges from Millipore (Marlborough, MA) to a resistivity of 18.2 $M\Omega \cdot cm^{-1}$ was used. 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) was purchased from Pierce (Rockford, IL).

Protein expression and purification

Recombinant wild-type P450cam protein was expressed in *Escherichia coli* and and purified as reported[1] and stored at −80°C at ~100 μM concentrations in 50 mM potassium phosphate buffer containing 150mM potassium chloride salt (pH 7.4), 200 μM camphor and 20 mM β-mercaptoethanol. Concentrations of cytochrome P450cam were determined using extinction coefficients ε_{391} = 102 $mM^{-1} \cdot cm^{-1}$ (camphor-bound) or ε_{417} = 115 $mM^{-1} \cdot cm^{-1}$ (substrate-free) in aqueous solution. The proteins were made substrate free by using Superdex G-25 columns.

Glass Substrate Preparation

Glass substrates were cleaned in piranha solution (1:3 30% H_20_2/H_2SO_4) for one hour at 80°C. Samples were cooled to room temperature and were then rinsed profusely with deionized (18.2 $M\Omega \cdot cm^{-1}$) water. Samples were then sonicated in 5:1:1 H_2O/NH_4OH/30% H_2O_2 and thoroughly rinsed with water. The samples were stored in deionized (18.2 $M\Omega \cdot cm^{-1}$) water prior to use.

Nanoparticle Preparation

Nanosphere lithography (NSL) was used to create monodisperse, surface-confined Ag nanoparticles. Polystyrene nanospheres (~2.2 μL) were drop-coated onto the glass substrates and allowed to dry, forming a monolayer in a close-packed hexagonal formation, which served as a deposition mask. The samples were then transferred to the evaporation chamber. The pressure in the vacuum chamber was maintained below 1×10^{-5} Torr during the evaporation. A silver film was evaporated on the slides. The deposition rate Ag was 1.0 ~ 1.5 Å/s. A Leybold Inficon XTM/2 quartz crystal microbalance (East Syracuse, NY) was used to measure the thickness of the Ag film deposited over the nanosphere mask, d_m. Following metal deposition, the samples were sonicated for 3-5 minutes in ethanol to the remove the polystyrene nanosphere mask. The perpendicular bisector of the nanoparticles was varied by changing the diameter of the nanospheres used. The height of the nanoparticles was varied by depositing varying amounts of Ag onto the sample. These two parameters were varied to alter the LSPR peak position throughout the visible region of the spectrum.

Nanoparticle Solvent Annealing and Functional Immobilization

For each experiment, the sample was stabilized and functionalized in a home built flow cell. Immediately following nanospheres removal, the samples were placed in 1 mM of 11-MUA

ethanol solution for 24 ~ 48 h. This time was determined to produce the repeatable and approximately full monolayer coverage of 11-MUA. After incubation, the nanoparticle samples were rinsed thoroughly with neat ethanol and dried by flowing N_2 gas through the sample cell. Samples were then activated using 10mM EDC and then they were incubated in 8 μM cytochrome P450cam for 1 h. After incubation, the nanoparticle samples were rinsed with MQ water and dried by flowing N_2 gas through the sample cell. Finally, the samples were incubated in 200 μM camphor buffer solution for 30 mins. After incubation, the nanoparticle samples were rinsed with MQ water and dried by flowing N_2 gas through the sample cell. These incubation times were determined to produce repeatable and approximately full monolayer coverage of the given analytes (data not shown).

Ultraviolet-Visible Spectroscopy

Macroscale UV-vis extinction measurements were collected using an Ocean Optics (Dunedin, FL) SD2000 fiber optically coupled spectrometer with a CCD detector and a Cary 300 Bio UV-vis spectrophotometer. All spectra in this study are macroscopic measurements performed in standard transmission geometry with unpolarized light. The extinction spectra of the same sample acquired from the two spectrometers have been tested to be consistent.

Control Experiments

1. Camphor-MUA adsorption

A control experiment was performed on MUA-functionalized Ag nanoparticles with camphor solution. Ag nanoparticles were fabricated with NSL (nanosphere diameter = 390 nm, metal thickness = 60 nm) and incubated in 1 mM MUA for 48 hrs. The sample was thoroughly rinsed with ethanol. Then, the extinction spectrum of MUA-functionalized Ag nanoparticles was collected in N_2 and shown in Figure 1 (black solid line). The extinction maximum was found to be 565.5 nm. The nanoparticles were then incubated in 200 μM camphor buffer solution for 30 mins and rinsed with MQ water. After the sample was dried with N_2, an extinction spectrum was measured and shown in Figure 1 (red dashed line). The extinction maximum stayed at the same wavelength at 565.5 nm. The constant LSPR position after introducing camphor to MUA-functionalized Ag nanoparticles indicates that camphor is unlikely to replace or adsorb to MUA.

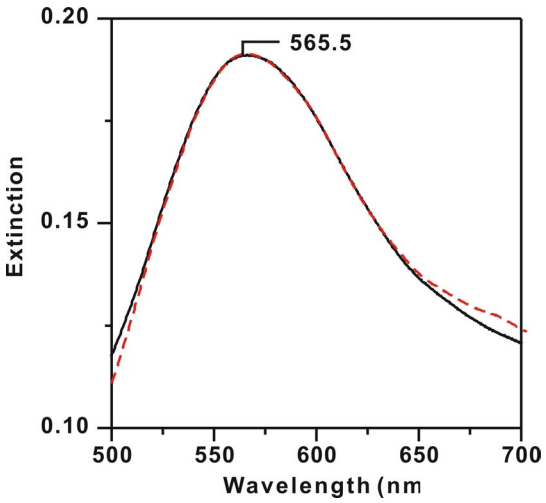

Figure 1 Camphor adsorption to MUA funcationalized Ag nanoparticles. Black solid line is the extinction spectrum of MUA-funtionalized Ag nanoparticles in N_2 before exposure to camphor; while red dashed line is after exposure to camphor. All the spectra were measured in N_2.

2. Camphor-bound CYP101 adsorption to MUA

Two sets of experiments have been performed following two different protocols. Figure 2A and 2B illustrate the scheme of protocol A and B. Protocol A is a 2-step experimental procedure of

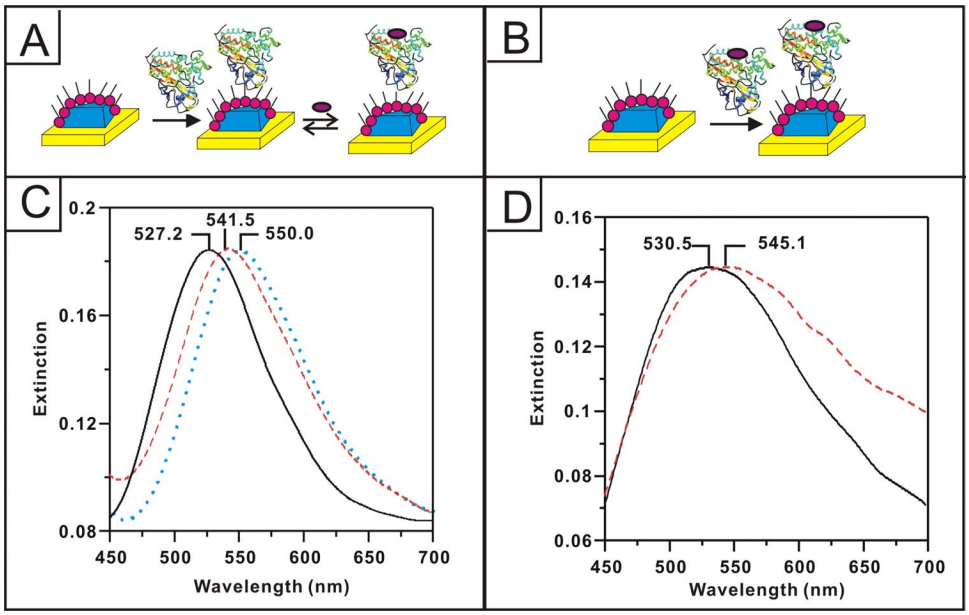

Figure 2 The LSPR shifts experiments following two different protocols. (A) Schematic illustration of protocol A. (B) Schematic illustration of protocol B. (C) Extinction spectra of functionalized Ag nanoparticles at each step following protocol A. Black solid line is the LSPR of MUA-Ag nanoparticles. Blue dotted line is the LSPR of the sample after exposure to camphor-free CYP101. Red dashed line is the LSPR of the sample after exposure to camphor. (D) Extinction spectra of functionalized Ag nanoparticles at each step following protocol B. Black solid line is the LSPR of MUA-Ag nanoparticles. Red dashed line is the LSPR of the sample after exposure to camphor-bound CYP101. All the

CYP101 binding to MUA, then camphor binding to CYP101. Protocol B is a 1-step procedure of camphor-bound CYP101 binding to MUA.

Figure 2C shows the extinction spectra at each step following protocol A. The initial LSPR of the MUA-functionalized Ag nanoparticles is located at 527.2 nm (solid black line) in N_2. After incubation in camphor-free CYP101, the LSPR shifts to 550.0 nm (blue dotted line). Lastly, after the sample was incubated in camphor solution, the LSPR blue-shifts by 8.5 nm to 541.5 nm (red dashed line) in N_2. This is a 14.3 nm red-shift compared to the initial LSPR.

Figure 2D shows the extinction spectra at each step following protocol B. The initial LSPR of MUA-functionalized Ag nanoparticles is at 530.5 nm (black solid line) in N_2. After incubation in camphor-bound CYP101, the LSPR of the nanoparticles shift to 545.1 nm (red dashed line) in N_2. That is a 14.6 nm red-shift.

In conclusion, adding camphor separately in the 2-step protocol A yields the same results as immobilizing the camphor-bound CYP101 onto the surface in the 1-step protocol B.

References:

1. Gunsalus, I. C.; Wagner, G. C. *Methods Enzymol.* **1978**, 52,166.