

## Supplementary Information

### **Biogenic production of palladium nanocrystals using microalgae and their immobilization on chitosan nanofibers for catalytic applications**

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#### **S1. Biosynthesis of Palladium Nanoparticles**

Wild type *Chlorella vulgaris* cultures (from the Australian National Algae Culture Collection at CSIRO, Tasmania) were used as the green microalgae source for the biosynthesis of palladium. Sterile algal freshwater media (MLA media)<sup>[1]</sup> containing standard micronutrients, nitrate, phosphate, carbonate buffer and vitamins was used as the substrate source. The microalgae cultures were mixed with Na<sub>2</sub>[PdCl<sub>4</sub>] solution at various concentrations (100, 50, 25, 12.5, 0 mg/L), towards reaching an initial total-chlorophyll content of around 1.8 mg/L (Figure 1a).

Na<sub>2</sub>[PdCl<sub>4</sub>] solution was prepared by dissolving Na<sub>2</sub>[PdCl<sub>4</sub>] powder (Sigma-Aldrich) in distilled and sterilized water overnight, and subsequently filtered through a sterile Pall<sup>®</sup> Acrodisc<sup>®</sup> 32 mm syringe filter (0.2 μm membrane) for further sterilization. Concentrations of Na<sub>2</sub>[PdCl<sub>4</sub>] in microalgae solutions are reported here as the initial concentrations measured by the gravimetric analysis before the filtration process. The experiments were conducted under batch conditions and cyclic diurnal conditions (16 h light/8 h dark) at a constant temperature (25 °C). Algae cultures (total liquid volume of 40 mL) were grown in 250 mL Erlenmeyer flasks, under continuous cool-white fluorescent illumination at an incident intensity of around 200 μmol photons m<sup>-2</sup>s<sup>-1</sup>(PAR) upon orbital shaking (Thermoline

Scientific) at 120 rpm.

Algal growth was investigated by measuring the total chlorophyll content (Chl a + Chl b) with respect to time, following the spectrophotometric method involving methanol extraction.<sup>[2]</sup> Chlorophyll content was also used to validate the viability of the cell cultures. All experiments were conducted in triplicates, with the standard deviation of each value given in the form of error bars within the related figure.

## S2. Characterization

A JEOL 2100 TEM instrument operating at 80 kV was used for determining the size and morphology of chitosan nanofibers and palladium nanoparticles. Samples were prepared by inserting the solutions on top of carbon-coated 200 mesh copper grids and allowing them to dry. High resolution images were obtained using a JEOL 3000F instrument operating at 300 kV. A Zeiss 1555 VP-FESEM with a 3 kV accelerating voltage was used to image samples coated with platinum (~3nm). XPS data was acquired using a VG ESCALAB220i-XL X-ray Photoelectron Spectrometer equipped with a hemispherical analyzer. The incident radiation was monochromatic Al K $\alpha$  X-rays (1486.6 eV) at 220 W (22 mA and 10kV). Survey (wide) and high resolution (narrow) scans were taken at analyzer pass energies of 100 eV and 50 eV, respectively. Survey scans were carried out over 1200-0 eV binding energy range with 1.0 eV step size and 100 ms dwell time. Narrow high resolution scans were run over a 20 eV binding energy range with 0.05 eV step size and 250 ms dwell time. Base pressure in the analysis chamber was  $4.0 \times 10^{-9}$  mbar and during sample depth profile analysis  $1.5 \times 10^{-7}$  mbar. A low energy flood gun (~6 eV) was used to compensate the surface charging effect. Argon ions at 3 keV beam energy were used to sputter off approximately 18 nm surface layers at a rate of ~3 Angstrom/second. The ion source gave a crater of approximately 3x3 mm. The energy calibration was referenced to the C 1s peak at 284.7 eV.

## S3. Cross Coupling Reactions

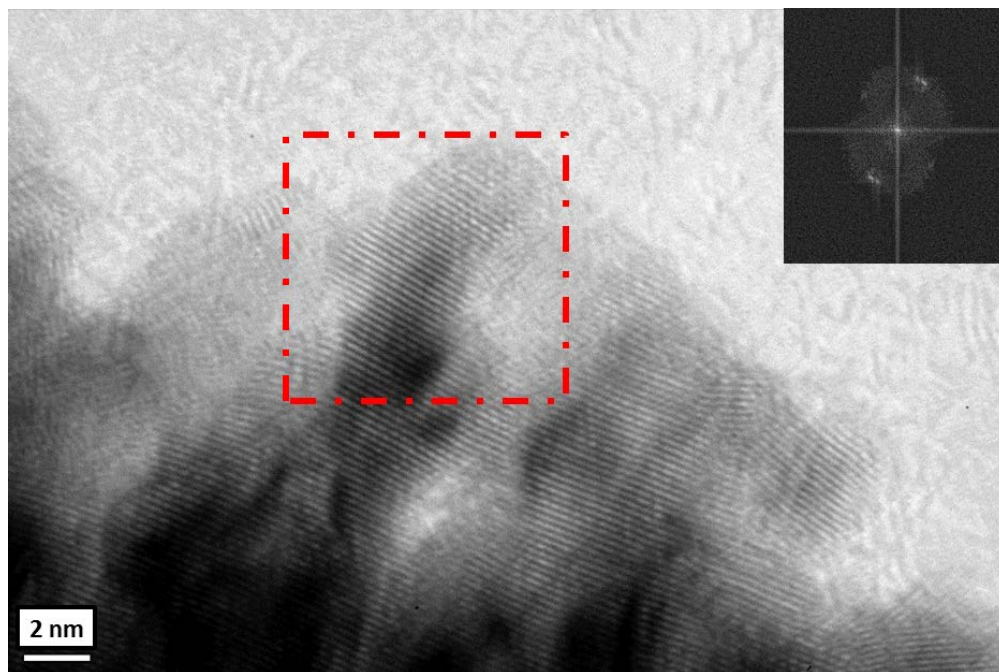
A rectangular piece of electrospun chitosan mat (3 x 2 cm per each piece) was placed into four week old 25 mg/L Na<sub>2</sub>[PdCl<sub>4</sub>] containing microalgae solutions (total volume: 40 mL per flask) for two weeks. The amount of palladium was established using inductively coupled plasma - optical emission spectroscopy (ICP-OES), at ~1.03 % w/w per each rectangular mat (3x2 cm). ICP-OES analysis was acquired with ARL-3520B sequential scanning ICP-OES,

with a 20mm torch, 1200W incident power, and a MDSN nebuliser. For the Mizoroki-Heck reactions iodobenzene (55  $\mu\text{L}$ ; 0.49 mmol), butyl acrylate (85  $\mu\text{L}$ ; 0.59 mmol), triethylamine (171  $\mu\text{L}$ ; 1.23 mmol) in dimethylformamide (DMF, 0.5 mL) were added to 6 hybrid palladium nanoparticle-chitosan mats with a total of 0.23% mol Pd per mol iodobenzene. The reaction mixture was heated to 80°C for 16 hours, whereupon the catalyst mat was filtered and recovered for its recycling after washing with DMF five times under nitrogen gas. Quantitative conversion yields were assessed gravimetrically upon the confirmation of the final product (butyl cinnamate) using  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy (Varian<sup>®</sup> 400 NMR).

#### **S4. Electrospinning of Chitosan**

Pd was collected by electrospun chitosan mat following the electrospinning procedure optimized by Bradshaw et al. (2011)<sup>[3]</sup>, which was slightly modified from the original protocol given by Ohkawa et al. (2004)<sup>[4]</sup>. The variables of electrospinning processes were as follows: (i) syringe pump speed: 0.1 mm/min, (ii) voltage: 18 kV, (iii) distance between the target and the tip of the syringe: 11 cm, (iv) target speed: 1 m/min, (v) traverse speed: 0.5 cm/min.<sup>[3]</sup> Chitosan (2-amino-2-deoxy-(1-4)- $\beta$ -D-glucopyranose), 75-85% deacetylated (Sigma-Aldrich), powder (6% wt) was mixed with TFA (trifluoroacetic acid): DCM (dichloromethane) solution (70:30 v/v), and stirred overnight for its complete dissolution. These ratios were taken to be optimum for attaining ultrafine chitosan nanofibers (Bradshaw et al. 2011).<sup>[3]</sup> TFA (trifluoroacetic acid) and DCM (dichloromethane) were obtained from Chem Supply. Before the electrospinning process, the mixture was sonicated for 15 min and 5.4% v/v glutaraldehyde (25% in H<sub>2</sub>O, Sigma-Aldrich) was immediately added to the chitosan/TFA/DCM solution for an effective crosslinking. Electrospinning of this cross-linked solution created insoluble nanofibers. Two rectangular pieces of electrospun chitosan mat (3 x 2 cm per each piece) were placed into one flask of (four weeks old) 25 mg/L Na<sub>2</sub>[PdCl<sub>4</sub>] containing microalgae solutions (total volume of 40 mL per flask) and left inside for two weeks. The amount of palladium was established by using ICP-OES, yielding ~1.03 % w/w per each rectangular mat (3x2 cm).

## S5. Reduction of Palladium with NADPH



**Figure S1.** High resolution TEM images of Pd(0) nanoparticles after the inoculation of  $\text{Na}_2[\text{PdCl}_4]$  precursor with an excess amount of NADPH, dissolved inside MLA algal growth-media,<sup>[1]</sup> indicating high crystallinity with (111) lattice constant of around 0.22 nm (inset: FFT pattern corresponding to the area shown with red rectangle).

## References

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