

## Supplementary Information

### **Ferritin-supported palladium nanoclusters: selective catalysts for aerobic oxidations in water**

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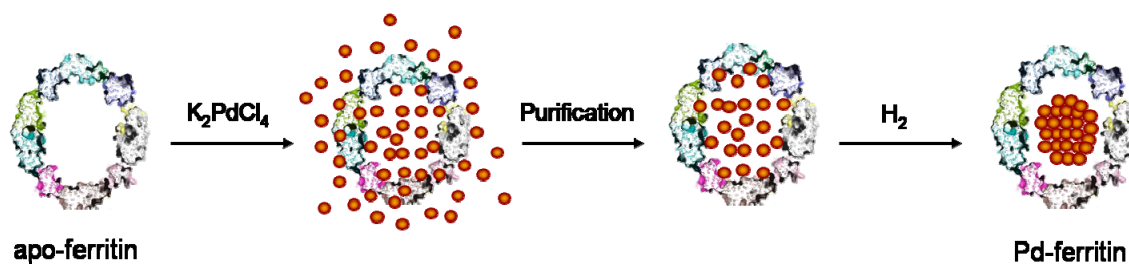
#### *Content:*

1. Experimental procedure:
  - a. Synthesis nano-Pd@PfFerritin
  - b. Characterisation nano-Pd@PfFerritin
  - c. Oxidation reactions
2. Recycling experiments

### 1a) Synthesis nano-Pd@PfFerritin (Scheme S1)

Ferritin and apoferritin were produced in our laboratories using a previously described procedure. Ferritin was dialysed against EDTA in order to remove residual iron.<sup>1</sup> Apoferritin (2.5 mg; MW = 440 kDa) was dispersed in a buffer solution of pH 8 (100 mM EPPS, containing 10 mM NaCl) and heated up to 60°C for 15 minutes. Then aliquots of K<sub>2</sub>PdCl<sub>4</sub> stock solution (40 mM) were added until a ratio of Pd/24mer of 250/1 was reached. The mixture was then stirred for another 10 minutes and cooled down to room temperature. After 30 minutes incubation at RT, the ferritin-Pd solution was further purified over a desalting column (PD-10, GE Healthcare) in order to remove non-bound Pd<sup>2+</sup> from the solution. The purified solution was de-aerated with argon and subsequently H<sub>2</sub> was added. For this purpose, 1 ml of a H<sub>2</sub>-saturated buffer solution was injected with a syringe. Upon addition, the yellowish catalyst solution turned deep brown due to nanoparticle formation. Samples were stored in closed vessels in the fridge, without special precautions.

Scheme S1. Preparation of Pd nanoclusters in the apoferritin cavity



### 1b) Nano-Pd@PfFerritin characterization

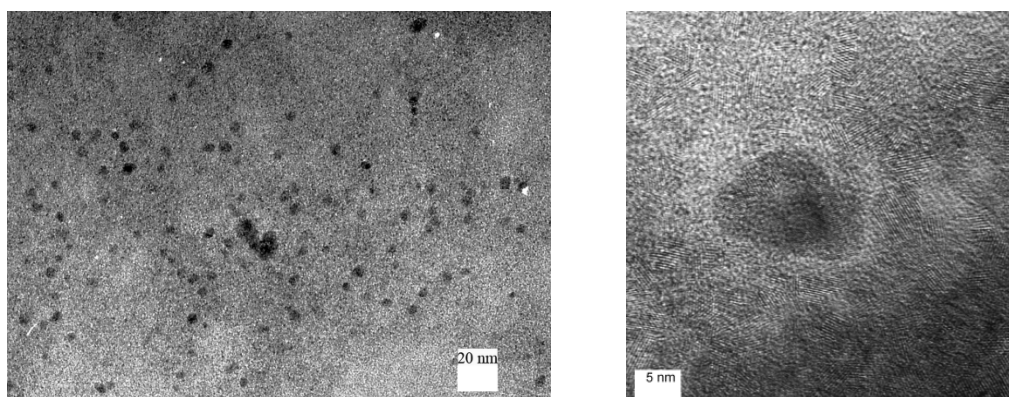
The catalyst samples from different production batches were analyzed by TEM and DLS. Pd-concentrations were determined from ICP-OES measurements. The protein concentration was determined by Bradford assay (typical concentration of protein was 5 mg/ml). Pd/ferritin ratios were determined by dividing Pd concentrations determined by ICP-OES (in the range of 116 to 187 µg/ml) and protein concentrations. From these measurements it turned out that values from 100 to 200 Pd atoms per protein cage were obtained depending on the batch.

*Dynamic Light Scattering* (DLS) was measured with a Zetasizer Nano ZS instrument (Malvern Instruments) in disposable PMMO cuvettes with 1 ml sample solutions. Samples diluted accordingly before measurements.

*High Resolution Transmission Electron Microscopy* (HR-TEM), was performed using a Philips CM30T electron microscope with a LaB<sub>6</sub> filament as source of electrons, operated at 300 kV. TEM measurements were performed in the TUDelft DCT/ NCHREM section.

The TEM images of the freshly prepared catalyst samples revealed homogeneously distributed palladium particles of  $5 \pm 1$  nm (Figure S1). 2 wt% uranyl acetate solutions were used for staining. In the zoom picture the darkest spot in the middle corresponds to the palladium, the lighter area is the ferritin shell.

Figure S1. TEM image of nano-Pd@Pfferritin (Pd/ferritin = 100/1) with uranyl acetate staining, scale bar 20 nm, zoom picture scale bar 5 nm.



### *1c) Oxidation reactions*

The reactions were performed at 30 bar pressure using 8% O<sub>2</sub>/N<sub>2</sub> mixture at 80°C in a polyblock reaction platform (Hastelloy C steel mini autoclaves, 16 ml capacity, manufactured by Hel Group). Mechanical stirring was performed at 800 rpm. Typical alcohol concentrations (all alcohols were commercial samples of >99% purity from Sigma-Aldrich) were in the range of 40 mM. In some cases biphasic mixtures are present because of the lower solubility of the substrates (1-phenyl ethanol, 3-methylbenzyl alcohol, 3-cyclohexenol, geraniol, 1-octanol and cyclohexanol). Ferritin concentrations are typically in the range of 1,3 μM. The starting Pd-ferritin solution was analyzed by ICP-OES. The corresponding palladium concentration is approximately 100 times (depending on the batch) higher in the range of 130 μM. No buffer was used during the experiments. MilliQ water was used to dilute the ferritin samples as prepared. Typical pH value of the solution was pH 8.

After the desired reaction time the reactors were cooled down to 20°C and depressurized. The reaction mixture was acidified with HCl and was extracted with diethyl ether and the internal standard (anisole) was added. Samples were analyzed by gas chromatography (Varian Star 3400Cx equipped with CP wax 52CB column). Calibration relative to anisole (internal standard) was performed using authentic samples of alcohols and aldehydes as reference. Mass balances for all samples were > 90% in all cases. The presence of benzoic acid was explicitly checked for the samples by extracting aqueous acidified solutions. These confirm the full selectivity for aldehyde production in the reaction.

## 2) Recycling experiments

The aqueous phase, after the extraction of reactants and products, was washed one more time with the organic solvent and was then concentrated by using centrifugal ultra-filtration tubes. The catalyst solution was used in a subsequent reaction without any further treatment.

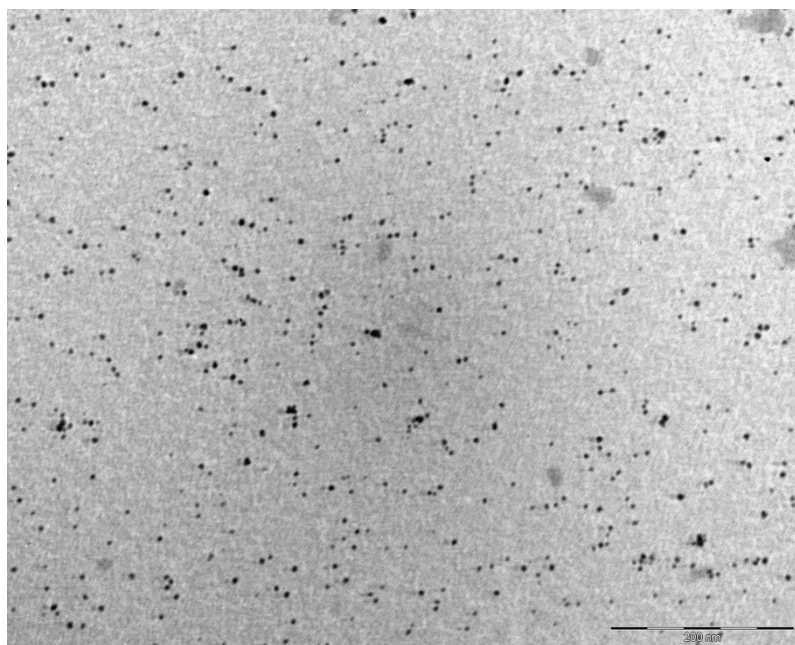
Table S1. Oxidation of 4-methoxybenzyl alcohol with nano-Pd@*Pf*Ferritin recycled from previous experiments.<sup>a)</sup>

| Cycle          | Yield 4-methoxy<br>benzaldehyde [%] |
|----------------|-------------------------------------|
| fresh catalyst | 74                                  |
| 1              | 74                                  |
| 2              | 73                                  |
| 3              | 40                                  |

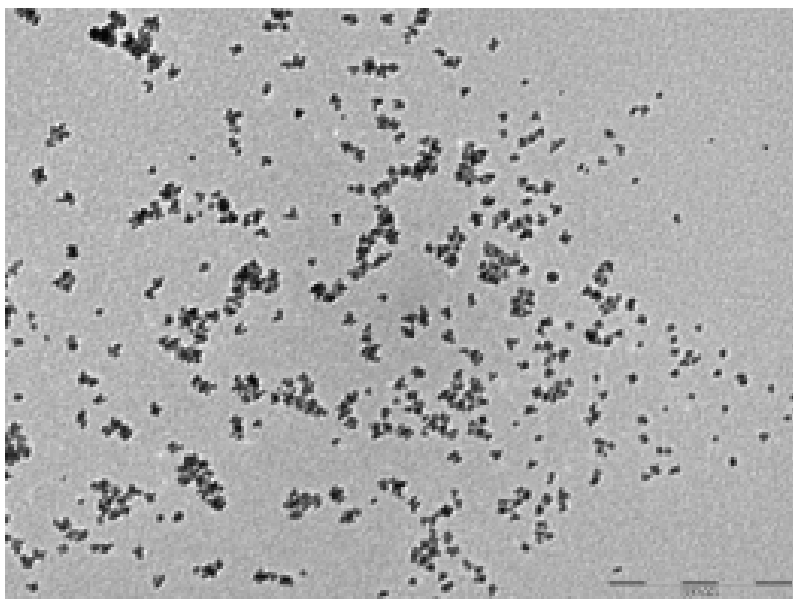
<sup>a)</sup> Conditions: 40 mM substrate, nano Pd@*Pf*Ferritin 1.3  $\mu$ M, 30 bar 8% O<sub>2</sub>/N<sub>2</sub> mixture, 80°C, 2 hours.

Figure S3. TEM micrographs for the nano-Pd@*Pf*ferritin, before (a) and after 1 reaction cycle with 4-methoxybenzyl alcohol (b). For experimental conditions, see Table S1. No staining was performed in this case. Loading of palladium 100 Pd/ferritin cage. Agglomeration of protein is observed after reaction, which however does not hamper the activity. Bars correspond to 200 nm.

a)



b)



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<sup>1</sup> J. Tatur, P. L. Hagedoorn, M. L. Overijnder, W. R. Hagen, *Extremophiles*, 2006, **10**, 139-148.