Supporting information

Synthesis of citrate-coated penta-twinned palladium nanorods and ultrathin nanowires with tunable aspect ratio

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1. Synthetic Procedure scale-up using Flexiwave Microwave Reactor

All the synthetic processes described in the main text were scaled up in a microwave reactor (Flexiwave Microwave Reactor), using the multi-vessel setup (15 vessels).

1.1. 10 nm Quasi-decahedron/ truncated octahedron Pd citrate-capped Nanocrystal Scale-Up method

Pd seeds (2 mL) were added to 30 mL of MilliQ water together with 79.5 μL of Pd (II) acetate (0.05 M) and 500 μL of a solution containing 0.34 M sodium citrate, 0.2 M formic acid and 0.5 mM L-ascorbic acid. This procedure was quickly repeated for 15 vessels that were then closed, moved in the microwave chamber and brought to 105 °C in 5 minutes. The reaction time was 10 minutes. The containers were then cooled to 20 °C.

1.2. Pd citrate-capped Nanorods Scale-Up method

Pd seeds (2 mL) were added to 30 mL of MilliQ water together with 79.5 μL of Pd(II) acetate (0.05 M), 900 μL of 0.5 M KBr and 500 μL of a solution containing 0.07 M sodium citrate, 0.1 M formic acid and 0.5 mM L-ascorbic acid. This procedure was quickly done for 15 vessels that were then closed, moved in the microwave chamber and brought to 105 °C in 5 minutes. The reaction time was 20 minutes. The containers were then cooled to 20 °C.

1.3. Pd citrate-capped Nanowires Scale-Up method

Pd seeds (2 mL) were added to 30 mL of MilliQ water together with 79.5 μL of Pd(II) acetate (0.05 M), 900 μL of 1 M KBr and 500 μL of a solution containing 0.07 M sodium citrate, 0.1 M formic acid and 0.5 mM L-ascorbic acid. This procedure was quickly repeated for 15 vessels that were then closed, moved inside the microwave chamber and brought to 105 °C in 5 minutes. The reaction was held stationary for 20 minutes and then gradually cooled to 20 °C.

2. Synthetic methods and TEM characterization

2.1. Pd Seed Synthetic Procedure

Pd seeds were synthesized by adding 80 μL of PdCl₂ acidic solution (56.4 mM) (Sigma-Aldrich) to 130 mL of MilliQ water at room temperature, immediately followed by a quick addition of 8.8 mL solution containing 0.03 M sodium citrate and 2 mM citric acid and 550 μL of freshly prepared NaBH₄ (0.02 M). The vessel was placed in glycerol bath already at 105 °C to obtain a quick reduction of the Pd ions. The reaction was kept at these conditions for 10 minutes under magnetic stirring at moderate rate. The vessel was then removed from the glycerol bath and left to cool under stirring for another hour.
**Figure S1.** BF-TEM images of Palladium seeds obtained with a 0.01 M of NaBH₄ (a) and (b), and seeds obtained with 0.02 M of NaBH₄ used for the synthesis of Nanorods and Nanowires (c) and size distribution analysis (d).

The BF-TEM image (a-d) demonstrates the effect of the concentration of the reducing agent: with a lower concentration of NaBH₄ we obtain seeds with a bigger dimension than the ones obtained with a two-times higher concentration of NaBH₄. Moreover, the seeds morphology changes with the concentration of NaBH₄. Higher concentration NaBH₄ likely favours the twinned morphology and, hence, the growth of nanowires.

### 2.2. Synthetic procedures and TEM images after synthesis and before any further purification

The syntheses were performed in a sealed glass container (ACE glass pressure reactor with Teflon cap). Palladium nanocrystals seeds (4 mL) were added to 60 mL of MilliQ water at room temperature together with 159 μL of Pd (II) acetate(0.05 M) and 1 mL of a solution containing 0.34 M sodium citrate, 0.2 M formic acid and 0.5 mM L-ascorbic acid. The concentration of KBr has been modified together with reaction time as specified in the caption (Figure S2-S7), in order to establish the role of bromide ions.

In all the syntheses with the presence of potassium bromide, two population of nanoparticles are obtained. One population consists of a mix of cubes, icosahedral and octahedral nanoparticles, and the other one consists of nanorods and nanowires. Therefore, a centrifugation is required in order to increase the percentage of nanorods/nanowires.
2.2.1. *Synthesis performed with 8 mM KBr*

![BF-TEM images of Pd nanorods and nanocubes prepared using 8 mM of KBr with a reaction time of 10 minutes, before the centrifugation.](image)

A population of Pd nanoparticles and one of nanorods are obtained (characterized by an average length of 38 nm and thickness of 6.5 nm).

2.2.2. *Synthesis performed with 13mM KBr*

![BF-TEM images of Pd nanorods and nanocubes prepared using 13 mM of KBr.](images)
Figure S3. BF-TEM images of Pd Nanorods, nanoparticles and nanocubes, prepared using 13 mM of KBr with reaction time of 10 minutes (A and B) and 20 minutes (C and D), before the centrifugation.

One population of Pd nanoparticles/ nanocubes together with one of nanorods are obtained. In A and B images, nanorods with an average length of 66 nm and thickness of 6.9 nm are shown. In C and D nanorods with an average length of 71 nm and thickness of 7.4 nm are shown.

2.2.3. Synthesis performed with 27 mM KBr

Figure S4. BF-TEM images of Pd Nanowires and nanoparticles, prepared using 27 mM of KBr with reaction time of 20 minutes (A and B) and 60 minutes (C and D) respectively, before the centrifugation.

One population of Pd nanoparticles/nanocubes together one of nanowires are obtained. In A and B images, nanowires with an average length of 280 nm and thickness of 7.5 nm are shown. In C and D images, nanorods with an average length of 470 nm and thickness of 7 nm are shown.
2.2.4. *Synthesis performed without KBr*

Figure S5. BF-TEM images of (A) Pd NPs formed by reduction of Pd(II) acetate in a solution containing 0.07 M sodium citrate, 0.2 M formic acid and 0.5 mM L-ascorbic acid, (B) Pd NPs in a solution containing 0.34 M sodium citrate, 0.42 M formic acid and 0.5 mM L-ascorbic acid.

BF-TEM images Figure S5 demonstrate that without the presence of KBr polydisperse in size and shape nanoparticles are obtained.

2.2.5. *Synthesis performed with 40 mM KBr*

Figure S6. BF-TEM images of polydisperse Pd nanoparticles synthesized by increasing the concentration of KBr to 40 mM.
2.2.6. **Synthesis performed with 54mM KBr**

![Figure S7. BF-TEM images of Pd nanoparticles obtained using 54 mM KBr.](image1)

With 40 mM of KBr (Figure S6), polydisperse in size and shape nanoparticles and nanorods are obtained. On the other hand, with 54 mM of KBr (Figure S7) aggregates of nanoparticles without any shape are obtained: this is probably due to the fact that the reduction rate of the Pd(II) precursor was nearly suppressed by excess of KBr.

![Figure S8. BF-TEM images of aggregated nanoparticles obtained in an open vessel (uncontrolled amount of oxygen in the reaction solution).](image2)

2.3. **Synthetic procedure performed with an open vessel**

The synthesis was performed in an open round bottom flask. Palladium nanocrystals seeds (4 mL) were added to 60 mL of MilliQ water at room temperature together with 159 μL of Pd (II) acetate(0.05 M) and 1 mL of a solution containing 0.34 M sodium citrate, 0.2 M formic acid and 0.5 mM L-ascorbic acid. The vessel was placed in a glycerol bath at room temperature and brought to 105 °C in 10 minutes. The reaction time was 20 minutes (under stirring at moderate rate).
2.4. **Synthetic procedure performed without the use of L-ascorbic acid**

The synthesis was performed in a sealed glass container (ACE glass pressure reactor with Teflon cap). Palladium nanocrystals seeds (4 mL) were added to 60 mL of MilliQ water at room temperature together with 159 μL of Pd (II) acetate (0.05 M) and 1 mL of a solution containing 0.34 M sodium citrate and 0.2 M formic acid. After 1 minute, 1.8 mL of 1 M KBr was added in the solution. The vessel was then sealed, placed in a glycerol bath at room temperature and brought to 105 °C in 10 minutes. The reaction time was 20 minutes (under stirring at moderate rate).

![BF-TEM images of Pd nanoparticles and nanowires, obtained without the use of ascorbic acid.](image)

Without ascorbic acid a higher polydispersity of nanowires in term of size and shape are obtained. Moreover, BF-TEM images show the presence of seeds that formed during the synthesis and did not grow.

2.5. **Synthetic procedure performed without sodium citrate**

The synthesis was performed in a sealed glass container (ACE glass pressure reactor with Teflon cap). Palladium nanocrystals seeds (4 mL) were added to 60 mL of MilliQ water at room temperature together with 159 μL of Pd (II) acetate (0.05 M), and 1 mL of a solution containing 0.2 M formic acid and 0.5 mM L-ascorbic acid. After 1 minute, 1.8 mL of 1 M KBr was added in the solution. The vessel was then sealed, placed in a glycerol bath at room temperature and brought to 105 °C in 10 minutes. The reaction time was 20 minutes (under stirring at moderate rate).

![BF-TEM images of Pd nanoparticles and nanowires, obtained without the use of ascorbic acid.](image)
Figure S10. BF-TEM images of Pd nanoparticles and nanowires, obtained without the use of sodium citrate.

Without sodium citrate, the nanoparticles, nanorods and nanowires tend to aggregate because they do not have any citrate molecule at the surface able to maintain colloidal stability.

2.6. Synthetic procedure performed under N₂

The synthesis was performed under N₂. Palladium nanocrystals seeds (4 mL) were added to 60 mL of MilliQ water at room temperature together with 159 μL of Pd (II) acetate (0.05 M) and placed under N₂. After degassing, 1 mL of a solution containing 0.34 M sodium citrate, 0.2 M formic acid and 0.5 mM L-ascorbic acid was added. After 1 minute, 1.8 mL of 1 M KBr was added in the solution. The vessel was placed in a glycerol bath at room temperature and brought to 105 °C in 10 minutes. The reaction time was 20 minutes (under stirring at moderate rate).

Figure S11. BF-TEM images of Pd nanoparticles and nanowires obtained in nitrogen atmosphere.

Under N₂ atmosphere BF-TEM images show one population of nanoparticles/nanocubes polydisperse in size and shape together with one of nanowires polydisperse in size.

2.7. Synthetic procedure with various concentration of formic acid

The synthesis was performed in a sealed glass container (ACE glass pressure reactor with Teflon cap). Palladium nanocrystals seeds (4 mL) were added to 60 mL of MilliQ water at room temperature together with 159 μL of Pd (II) acetate (0.05 M) and 1 mL of a solution containing 0.34 M sodium citrate, 0.5 mM L-ascorbic acid and different concentration of formic acid (Figure S12-S14). The vessel was placed in a glycerol bath at room temperature and brought to 105 °C in 10 minutes. The reaction time was 20 minutes (under stirring at moderate rate).
2.7.1. *Synthesis performed without the presence of formic acid*

Figure S12. BF-TEM images of Pd nanoparticles obtained without the use of formic acid.

2.7.2. *Synthesis performed with 0.1 M Formic acid*

Figure S13. BF-TEM images of Pd nanoparticles, nanorods and nanowires obtained with 0.1 M formic acid.

2.7.3. *Synthesis performed with 0.4 M Formic acid*

Figure S14. BF-TEM images of Pd nanoparticles, nanorods and nanowires obtained with 0.4 M formic acid.
Without formic acid polydisperse Pd nanoparticles in term of size and shape are obtained. The images in Fig S12 demonstrate the need of synergistic interplay of KBr and formic acid allow to start the anisotropic growth. In the case of 0.4M of formic acid BF-TEM image (Figure S14) shows a population of nanoparticles polydisperse in shape and size together with one of 280 nm nanowires. With 0.4M of formic acid there is no change in shape and size, only an excess of formic acid. Instead 0.1M BF-TEM image (Figure S13) shows a population of polydisperse nanorods together with one of unshaped nanoparticles, that demonstrate that concentration is not sufficient to obtain ultrathin nanowires.

2.8. Synthetic procedure performed without the use of seeds

The synthesis was performed in a sealed glass container (ACE glass pressure reactor with Teflon cap). In a vessel containing 64 mL of MilliQ water, at room temperature, 159 μL of Pd (II) acetate (0.05 M) and 1 mL of a solution containing 0.34 M sodium citrate, 0.5 mM L-ascorbic acid and 0.2 M formic acid were added. The vessel was placed in a glycerol bath at room temperature and brought to 105 °C in 10 minutes. The reaction time was 60 minutes (under stirring at moderate rate).

Figure S15. BF-TEM images of Pd nanoparticles and nanowires prepared without the use of Pd seeds (A and B). Size distribution analysis of length and thickness for Pd nanowires are shown in (C) and (D), respectively.

Without seeds, very polydisperse in length nanowires with an average thickness of 15 nm are obtained, as is shown in Figure S15 C. The small Pd seeds are necessary to obtain ultrathin and homogeneous in length penta-twinned nanowires.
2.9. Table of physical-chemical parameters changed during the syntheses and results obtained

| Palladium Np                            | KBr (mM) | Diameter (nm) | Length (nm) | Reaction time (min) | Aspect ratio |
|-----------------------------------------|----------|---------------|-------------|---------------------|--------------|
| Unshaped                                | 54       | n.a           | n.a         | 20                  | n.a          |
| Polydisperse                            | 40       | n.a           | n.a         | 20                  | n.a          |
| Nanowires                               | 27       | 7             | 470         | 60                  | 67.2         |
| Nanowires                               | 27       | 7.5           | 280         | 20                  | 37.3         |
| Nanorods                                | 13.5     | 7.4           | 71          | 20                  | 9.5          |
| Nanorods                                | 13.5     | 6.9           | 66          | 10                  | 9.5          |
| Nanorods                                | 8        | 6.5           | 38          | 10                  | 5.8          |
| Quasi-decahedron/truncated octahedron   | 0        | 8.9           | 8.9         | 20                  | 1            |

3. Cytotoxicity assays

HeLa cells (human cervix epithelioid carcinoma cells, ECACC), were expanded at 37 °C in a humidified incubator containing 5% CO₂ in high glucose DMEM (Sigma-Aldrich) supplemented with 10% (v/v) FBS (Sigma-Aldrich), 100 U/mL penicillin and 100 mg/mL streptomycin (Sigma-Aldrich).

3.1. Cell viability assessment by MTS assay

Viability of HeLa cells was measured using MTS(3-(4,5-dimethylthiazol-2-yl)-5-(3-cyrtboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium, salt) assay obtained as Cell-Titer96 Aqueous One Solution from Promega. The assay was performed according to the manufacture’s protocol. HeLa (10000 cells/well) cells were plated in a 96-well tissue culture plate (Constar) in a final volume of 100 μL and incubated for 24 hours at 37 °C and in an atmosphere with 5% CO₂. The culture medium was then removed and replaced with DMEM 10% FBS containing respectively palladium nanowires and nanorods at concentrations of 5, 10 and 25 μg/mL for 24 and 48hours. Non-treated cells were used as a control. After the incubation, the cell medium was removed, replaced by 120 μL MTS working solution (20 μL MTS reagent plus 100 μL of cell culture medium) and incubated with cells for 2 hours at 37 °C and 5% CO₂. A Biotek Synergy HT plate reader was used. The absorbance at 490 nm wavelength was measured using the plate reader. The absorbance value of MTS working solution diluted in cell culture medium was taken as blank. Cell viability was calculated by subtracting the absorbance blank from absorbance samples. Data were normalized to the control samples (non-treated cells) and expressed as mean ± SD. All experiments were done in quadruplicates.
Figure S16. Cell viability of Hela cells assessed with MTS assay after 24 and 48 h of incubation with Pd nanorods and Pd nanowires at concentration of 5, 10, 25 µg/mL. Data are normalized to the values of the untreated samples, set to 100%. The error bars represent the standard deviation.

### 3.2. Lactate dehydrogenase leakage (LDH) assay

HeLa (10 000 cells/well) cells were seeded in 96-well microplates in a final volume of 100 µL and incubated in an atmosphere with 5% CO$_2$ at 37 °C. After 24 hours, the culture medium was removed and cells were treated with nanorods and nanowires at concentrations of 5, 10, and 25 µg/mL respectively for 24 and 48 hours. Non-treated cells were used as a control. Assay plate was removed from 37 °C incubator and equilibrate to 22 °C approximatively 20 minutes before the test. Afterwards, the LDH leakage assay was performed as per manufacturer’s instructions for the CytoTox-ONE homogeneous Membrane Integrity Assay reagent (Promega). Positive control samples were performed adding 2 µL of lysis solution, provided by the kit and blank samples were prepared mixing 100 µL of CytoTox-ONE™ mix with cell-free culture medium. The plate was incubated at 22 °C for 10 minutes. 50 µL of Stop solution were added to each well and the 560-590 wavelength absorbance was measured using the plate reader (Biotek Synergy HT plate reader). Plasma permeability was calculated as (absorbance samples – absorbance blank). Data were normalized to negative control samples and were expressed as mean ± SD.
Figure S17. Membrane integrity assay on HeLa cells after Pd nanorods and Pd nanowires incubation for 24 and 48h; positive controls were obtained with lysis solution provided by the kit. Data are normalized on positive control samples expressed as 100% and reported as the average ± standard deviation.

4. Peroxidase-like activity

A systematic study on palladium nanowires and nanorods was performed to analyze their (peroxidase) antioxidant nanozyme properties. In order to investigate the HRP-mimicking activity, 3,3′,5,5′-tetramethylbenzidine (TMB) and hydrogen peroxide (H₂O₂) were chosen as chromogenic substrate. The TMB oxidation reaction kinetics has been characterized by UV-Vis spectroscopy measurement (absorbance peak at 652 nm). The reaction kinetics was monitored during the time. The HRP-like activity test was performed at room temperature under acid pH for 10 mM Acetate buffer at pH 4.7 and 100mM H₂O₂, necessary to operate. 1ppm nanowires, 1ppm nanorods and 0.1 ppm Pt NPs activities are assessed in the experiment.

Figure S18. UV-vis absorption–time curves of the TMB–H₂O₂ reaction system catalyzed by (circle symbol) Pd nanowires at concentration of 1 ppm, (triangle symbol) Pd nanorods at concentration of 1 ppm and (square symbol) spherical platinum nanoparticles at concentration of 0.1 ppm.