Supporting Information

In Vivo and *in Vitro* Protein Mediated Synthesis of Palladium Nanoparticles for Hydrogenation Reactions

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Experimental Section

Chemicals. All chemicals were used as received. $PdCl_2$ (7647-10-1) was purchased from Strem Chemicals. Isopropyl-beta-D-thiogalactopyranoside (IPTG) and Kanamycin sulfate were purchased from Sigma-Aldrich. $NaBH_4$ (16940-66-2), yeast extract and tryptone were bought from Merck. Analytical grade chemicals such as HCl and NaOH were purchased from Bio-Lab Chemicals. Restriction and ligation enzymes and PCR reagents were purchased from New England BioLabs. DNA extraction and isolation of plasmids kits were purchased from MACHEREY-NAGEL.

Bacterial strain cultivation. Bacterial cells were grown in Luria-Bertani (LB) broth (tryptone, yeast extract, NaCl [10, 5, and 5 g/liter, respectively]) at 37°C unless mentioned otherwise. Kanamycin (50 mg/liter) was added when needed. A single colony of the *E. coli* BL21 (DE3) containing the plasmid described above was inoculated into 5ml of LB media. The cells were grown at 37°C overnight with shaking at 1800 rpm. The next morning the overnight culture was transferred into 50ml and later into 500ml fresh medium to permit exponential growth. When the OD_{600} value reached ~0.6, protein expression was induced by addition of 0.1 mM IPTG, followed by prolonged growth at either 23°C. Cells were then harvested by centrifugation at 4,500 rpm for 35min and resuspended in phosphate buffer (PB) 10mM pH=7.4.

Protein Purification. The Precipitated cells were resuspended with an additional solution of fresh 20 mL PB. The lysis was performed using a Qsonica Q500- 500W sonicator (Lumitron), 5 sec on, 5 sec off cycles for 20 min total time on. (1/2" probe, 40% amplitude). The cell lysate was then centrifuged twice for 20 min (10,000 rpm, 4°C). The protein was purified using immobilized metal affinity chromatography (1 mL, High-affinity Ni-NTA resin, GenScript), when the supernatant was loaded on the affinity Ni-charged resin. The column was washed with 15 column volumes of wash buffer (0.1M PB, 500mM NaCl, 50mM imidazole, pH 7.4) to remove any loosely bonded proteins. Afterwards, the 6His-SP1 was eluted using 3 column volumes of elution buffer (0.1M PB, 500mM NaCl, 500mM imidazole, pH 7.4). In order to remove excess imidazole, the eluted 6His-SP1 solution was dialyzed overnight in dialysis membrane (MWCO 12-14kDa, REPLIGEN) and filtered using a 30kDa centrifuge filter (Amicon Ultra-4, Mercury) at 4,200 rpm for 20 min and 4°C, then 6His-SP1 was resuspended in 0.1M

PB, pH 7.4. Purified 6His-SP1 was stored in 4°C in 500 µL aliquots. Protein concentration was determined using UV-Vis spectroscopy at 280nm and the purities were analysed based on Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) gels (SurePAGE, Bis-Tris 4-20%, GenScript). Prior to loading, samples were mixed with SDS–PAGE sample buffer containing SDS and β-mercaptoethanol and heated at 95°C for 10min to facilitate denaturation and reduction of proteins. Proteins were detected using Coomassie blue staining.

***in vitro* synthesis of Pd-NPs**. In order to determine the desired ratio between the palladium ions and the protein in the solution, we used the following calculation, assuming spherical 2.7nm Pd-NPs in diameter inside the protein's cavity (Equation S1):

$$ratio = \frac{Volume\ of\ one\ NP}{Volume\ of\ Pd\ atom^{1}} = \frac{\frac{4}{3}\pi(\frac{2.7 \cdot 10^{-9}m}{2})^{3}}{14.614 \cdot 10^{-3}\frac{nm^{3}}{atom} \cdot \frac{1m^{3}}{10^{27}nm^{3}}} = 705.4 \approx 700\frac{Pd\ atoms}{nanoparticle}$$

Therefore, the ratio between the 12-mer protein and the palladium salt was 1:700. 20mM stock solution of $PdCl_2$ was prepared as follows: 10mg of $PdCl_2$ was dissolved in 356µL 1M HCl and sent for bath sonication for 10min. ddW was then added to volume of 2.81ml and pH was adjusted to 7.4 by slow titration of 1M NaOH (400µL). 6His-SP1 (0.7µM) was incubated overnight with 0.5mM $PdCl_2$ salt, and excess of $NaBH_4$ (0.12 mg/ml) was added to the solution as a reducing agent. The formed Pd-NPs were characterized by dynamic light scattering (DLS), Zeta potential (Zetasizer Nano ZS, Malvern Panalytical), TEM microscopy (as described below) and by UV-Vis absorbance spectrum using a plate reader (Synergy H1, BioTek). To test the stability of the Pd-NPs, samples were incubated at RT for 9 days and characterized by the same methods.

TEM analysis of Pd-NP. Transmission Electron Microscopy (TEM) analysis was performed using Bio-twin T12. The samples were prepared on a carbon coated copper grid by dropping 5µL of the sample on grid. Extra solution was removed by a blotting paper. For protein staining, 5µL of Uranyl acetate 1% was used. After extra solution removing, the film was allowed to dry in RT. Samples kept in vacuum until readings were taken.

DNA manipulations. DNA extraction and isolation of plasmids were performed according to the manufacturer's protocols. Restriction of DNA, ligation of DNA fragments, separation of DNA fragments by agarose gel electrophoresis and transformations were performed according to the protocols of Ausubel *et al*[1].

Construction of modified PdBP-SP1. The plasmid pJexpress 411 T7 Kan (ATUM) containing the ORF of 6His-SP1 (including restriction sites of NdeI and BamHI in its N-terminus and C-terminus regions, respectively) was kindly given by Oded Shoseyov's group, the Hebrew university of Jerusalem. The plasmid was purified from *E. coli* BL21 (DE3) cells, then amplified via overhang PCR procedure with forward primer containing the sequence that encodes the palladium binding peptide SVTQNKY (as shown in Table S1) to be translated in the N-terminus region of the protein. The forward and reverse primers contained the restriction sites of NdeI and BamHI, respectively. The PCR product was digested with the appropriate restriction

enzymes, then was extracted from agarose gel. Purified pJexpress 411 plasmid was digested as well with the same enzymes. Next, the digested PCR product was ligated with the digested plasmid and transformed to *E. coli* DH5α competent cells by heat shock. The cells were grown on selective agar plates (containing kanamycin) and single colonies were picked. In order to verify the correct insertion of the PdBP sequence to the plasmid, specific primers were designed for colony-PCR assay (Table S1). Colonies that produced PCR products were grown in LB media and PdBP-SP1 plasmids were purified and sent for Sanger sequencing. The verified plasmid was transformed to *E.coli* BL21 (DE3) competent cells.

Table S1. Oligonucleotides used in this study.

Oligonucleotides	Sequence (5'-3')[a,b]	Restriction site
FWPdBP-SP1 (overhang PCR)	GATACATATG**AGCGTGACCCAGAACAAATAT**GCAACCAGAACTCCAAAACTTGTGAAGCACACATTG	NdeI
RVPdBP-SP1 (overhang PCR)	GCTTGGATCCTTAGTAGAGAAAATAGTCTATCACAAGACGCTGTGACAAAGTAGGCAAAAACCC	BamHI
FWPdBP-SP1 (colony PCR)	G**AGCGTGACCCAGAACAAATAT**GC	-
RVPdBP-SP1 (colony PCR)	GCTGTGACAAAGTAGGCAAAAACCC	-

[a] Introduced restriction sites are underlined.
[b] PdBP sequence is shown in bold letters.

***in vivo* biosynthesis of Pd-NPs**. *E. coli* BL21 (DE3) containing the plasmid pJexpress 411 (T7) with either *6His-SP1* or *PdBP-SP1* ORF were grown to OD_{600}=0.6. Cells were induced with 0.1mM IPTG for 4 hours at 30˚C, then the bacteria were centrifuged to remove the growth media. The bacteria were resuspended with 10mM PB pH=7.4 and were incubated overnight with 2mM $PdCl_2$ salt. Residues of $PdCl_2$ salt were then washed, and the cells were further incubated for three days in 37^{o}C and 180rpm in shaker incubator. In each experiment, three control groups were examined as follows: cells without $PdCl_2$ salt, cells without plasmid and cells without overexpression. Each group was tested in duplicates. After three days of incubation, darker colour was observed in the plasmid-containing bacteria, indicating the formation of Pd-NPs in the cells (Figure S7). The palladium content inside the cells was determined by inductively coupled plasma optical emission spectrometry (ICP-OES, Agilent 5100 VDV) measurements as follows: 5ml of *E.coli* BL21 6His-SP1 (IPTG+) cells were harvested after three days of incubation and transferred to 7ml vials. The vials were centrifuged at 7000rpm and supernatants were discarded. Cell pellets were incubated at 400^{o}C for 2.5hr and resulted ash was resuspended in 5ml 7% nitric acid using ultrasonic bath at 40^{o}C for 1hr. Standard calibration curves for ICP-OES were prepared using 0-100 ppm Pd standard stock solutions (Merck) prior to the analysis of the cells. The intensity was monitored in triplicates in different dilutions. X-Ray Photoelectron Spectroscopy (XPS) measurements were performed in an analysis chamber (UHV – 210^{-10} Torr during analysis) using a Versaprobe III – PHI Instrument (PHI, USA). The sample was irradiated with a Focused X-Ray AlKα monochromated X-rays source (1486.6eV) using an X-Ray beam (size 200micron, 50W, 15kV).

The outcoming photoelectrons were directed to a Spherical Capacitor Analyzer (SCA). The sample charging was compensated by a Dual Beam charge neutralization based on a combination of a traditional electron flood gun and a low energy argon ion beam. HR-XPS analysis was performed with freeze dried *E.coli* BL21 6His-SP1 (IPTG+) bacterial cells after the synthesis of PdNPs to identify the oxidation state of the PdNPs. HR-XPs data were collected with a Pass Energy of 112eV (Step size = 0.1eV) and a Dwell Time of 20ms.

Hydrogenation Measurements. Cells after 3 days of incubation after washing from excess $PdCl_2$ were taken for hydrogenation measurements. In a typical procedure, hydrogen (1.23µmol) and acetylene (0.614µmol) gases were incubated in sealed Argon purged vials containing 400µL bacteria and a magnetic stirrer (conducted in triplicates). The Amounts of acetylene and the generated ethylene and ethane were monitored by Gas Chromatograph equipped with flame ionization detector (GC-FID) using BP-Bond-Q column. In each timepoint, 50µL from the vial's headspace were injected to the column using a 100µL syringe. Ethylene percentage in each timepoint was calculated from the acquired chromatograms as follows (Equation S2):

$$\%Ethylene = \frac{Ethylene\ Area}{Acetylene\ Area + Ethylene\ Area + Ethane\ Area} \cdot 100\%$$

Reusability of the biosynthesized Pd-NPs in catalysis was measured for the *E.coli* BL21 6His-SP1 (IPTG+) group in five sequential cycles (in quadruplicates, Figure S9). At the end of each reaction, samples were purged with Argon gas and resealed. To check the cell's viability after the fifth cycle, samples were picked by a sterile loop and spread on LB-agar plates (Figure S10a). In order to eliminate an option of contamination, the expression of 6His-SP1 was verified using colony PCR as described above. Indeed, a typical ~350bp band was observed (Figure S10b).

Turnover frequency (TOF) calculation. The TOF for each cycle in the reusability test was determined by calculating the slope of mol ethylene/mol PdNPs as a function of time in hours, considering the following assumptions: (i) the amounts of generated ethylene can be estimated by multiplying the ethylene percentage with the initial acetylene content. (ii) The size of the biosynthesized PdNP is 2.7nm, thus one PdNP is consisted of 705.4 Pd atoms, as demonstrated in a previous section and confirmed by cryo-TEM images (Figures 3c-e, S11). (iii) The bacterial concentration for *E.coli* BL21 6His-SP1 (IPTG+) after PdNPs biosynthesis is estimated as $6.1 \cdot 10^8 \frac{CFU}{ml}$, as shown in Figure 3a. (iv) ICP-OES measurements revealed that the palladium concentration inside the cell (marked as $C_{Pd,final}$) after the biosynthesis process was (49.7±3.8)ppm. Thus(Equation S3):

$$\frac{mol\ product}{mol\ catalyst} = \frac{mol\ acetylene \cdot \%ethylene}{mol\ PdNPs} = \frac{mol\ acetylene \cdot \%ethylene}{\frac{C_{Pd,\,final} \cdot V_{solution}}{Mw\ (Pd) \cdot \frac{\#Pd\ atoms}{particle}}}$$

Moreover, we calculated the number of biosynthesized PdNPs per bacterial cell as follows(Equation S4):

$$\frac{\frac{C_{Pd,\,final}\cdot V}{Mw\,(Pd)}\cdot N_{AV}}{C_{cells}\cdot V\cdot\frac{\#Pd\ atoms}{particle}}=\frac{\frac{49.74\frac{mg}{L}}{106420\frac{mg}{mol}}\cdot 6.022\cdot 10^{23}\frac{atoms}{mol}}{6.1\cdot 10^{8}\frac{CFU}{ml}\cdot\frac{705.4Pd\ atoms}{PdNP}\cdot 1000\frac{ml}{L}}=\boxed{654{,}121\frac{PdNPs}{bacteria}}$$

Cryo-TEM analysis for whole cell imaging. Cryo-TEM was carried out using a FEG-equipped FEI Talos 200C high-resolution transmission electron microscope operating at 200kV. Samples were transferred using a transfer station to a Gatan 626 cooling holder and introduced into the microscope. Sample preparation: 6 µL of sample solution was applied to a copper grid coated with holey carbon within Controlled Environment Vitrification System (CEVS) and was blotted at 25°C. The grid was then plunged quickly into liquid ethane (-183°C).

Cell viability measurements. Bacterial colony forming units (CFU) were determined as follows: the cells were diluted and spread in 3 different dilutions (10^{-4},10^{-5},10^{-6}) in duplicates after overnight incubation with $PdCl_2$ (t=0) and after 3 days (excess of salt was removed, t=3 days). The diluted cells were spread on LB-agar plates, and plates with 30-300 colonies were counted for CFU/ml calculation as described below (Equation S3):

$$\frac{CFU}{ml}=\frac{\#\ Colonies}{(Volume\ of\ culture\ plate)\cdot(Dilution\ factor)}$$

Supplementary Results

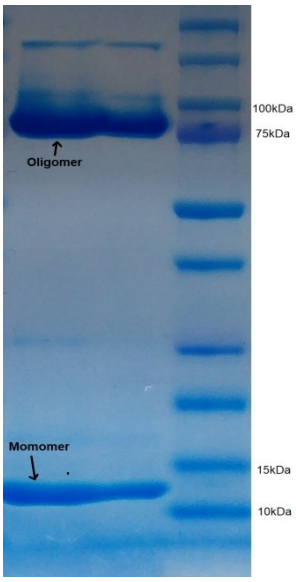

Fig. S1 SDS-PAGE analysis of purified 6His-SP1 protein. The "oligomer" band corresponds to the hexamer of SP1.

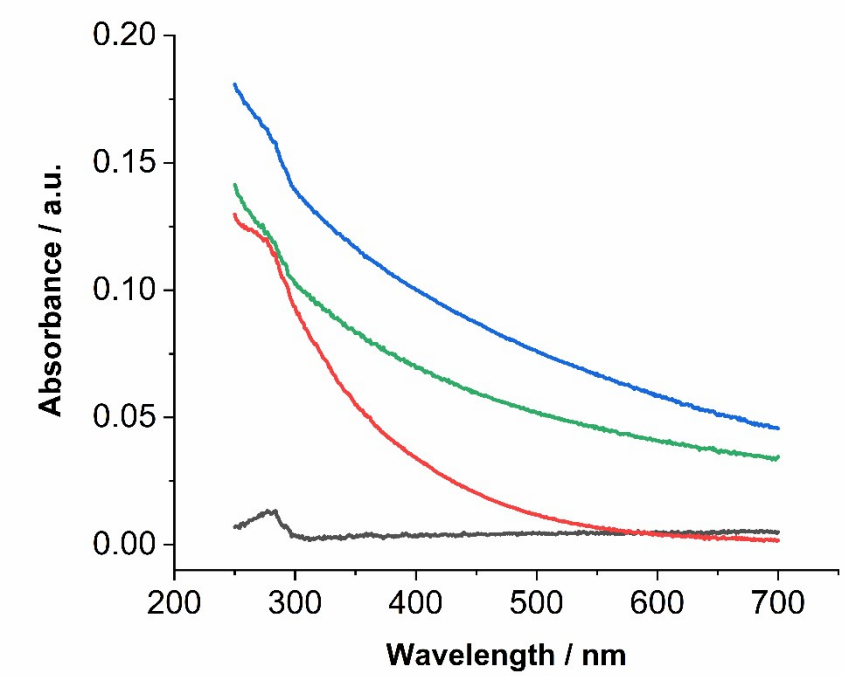

Fig. S2 UV-Vis absorption spectra of the *in vitro* synthesized PdNPs. Gray – 6His-SP1 protein. Red – 6His-SP1 with $PdCl_2$ salt before reduction. Blue and green – PdNPs stabilized by 6His-SP1 immediately after reduction and after 9 days at RT, respectively.

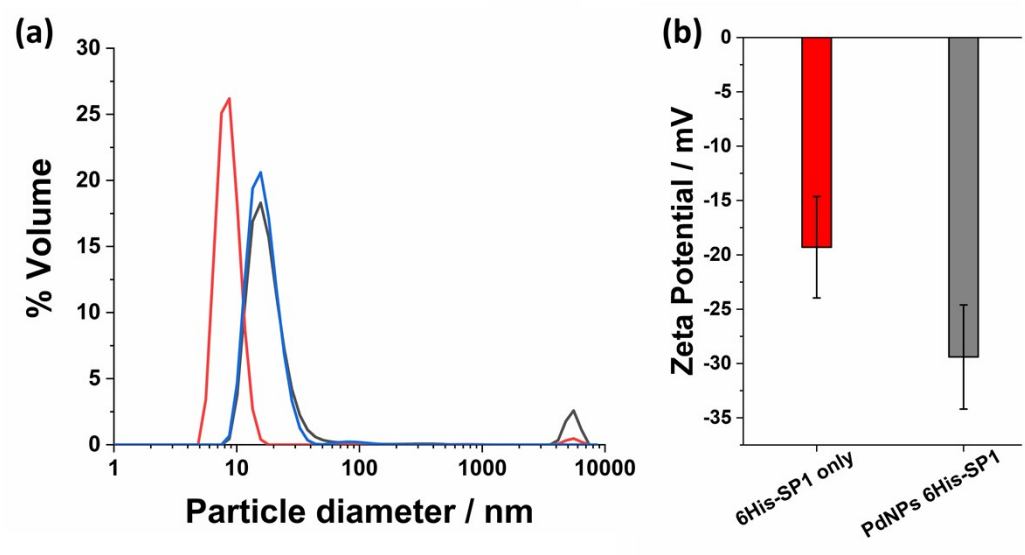

Fig. S3 Characterization of the formed Pd-NPs in the presence of the protein 6His-SP1. (a) Particle size distribution of 6His-SP1 only, PdNPs stabilized with 6His-SP1 and PdNPs stabilized with 6His-SP1 9 days after formation (red, grey and blue curves, respectively).

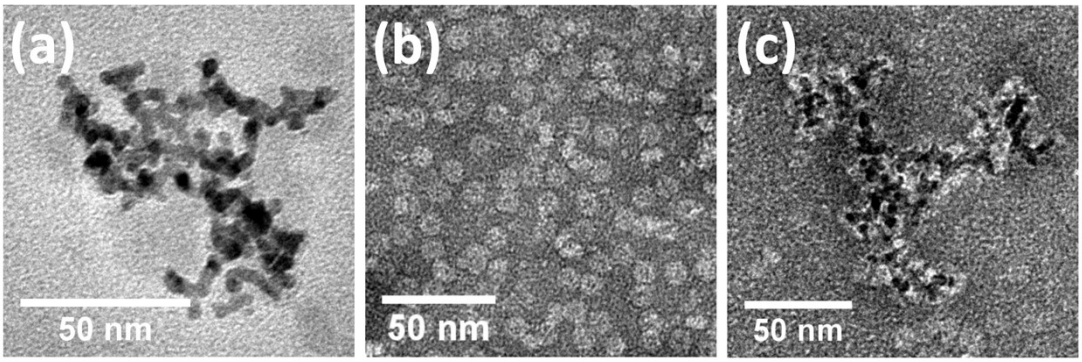

Fig. S4 *in vitro* synthesis of Pd-NPs. (a) and (c) TEM images of the formed Pd-NPs in the presence of the protein 6His-SP1 without and with negative staining, respectively. (b) TEM image of purified 6His-SP1.

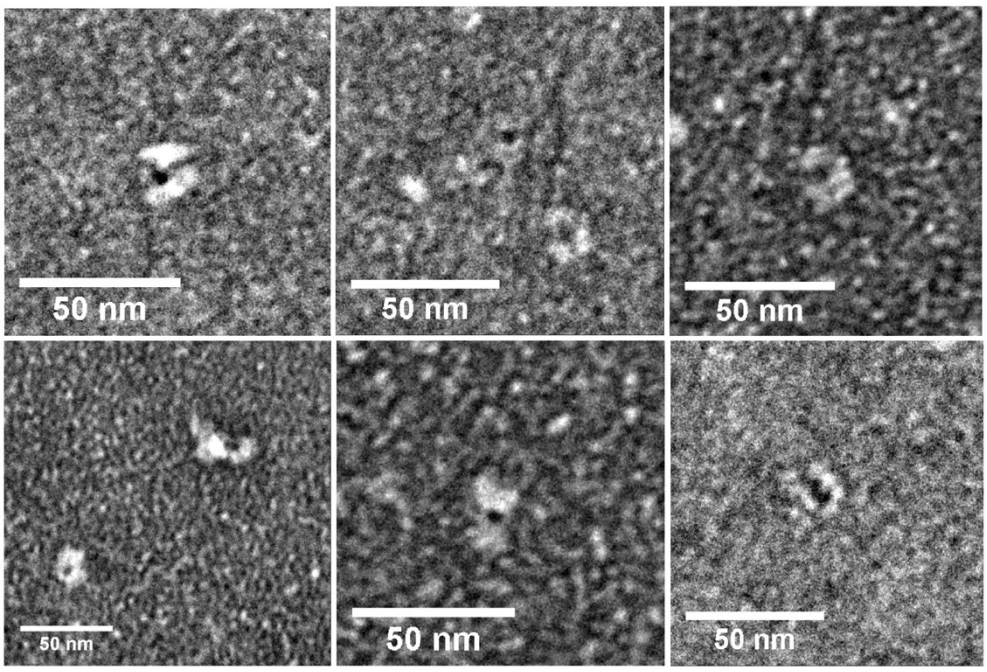

Fig. S5 TEM images of the formed Pd-NPs in the presence of the protein 6His-SP1 after 9 days of incubation at RT, with negative staining (1% uranyl acetate).

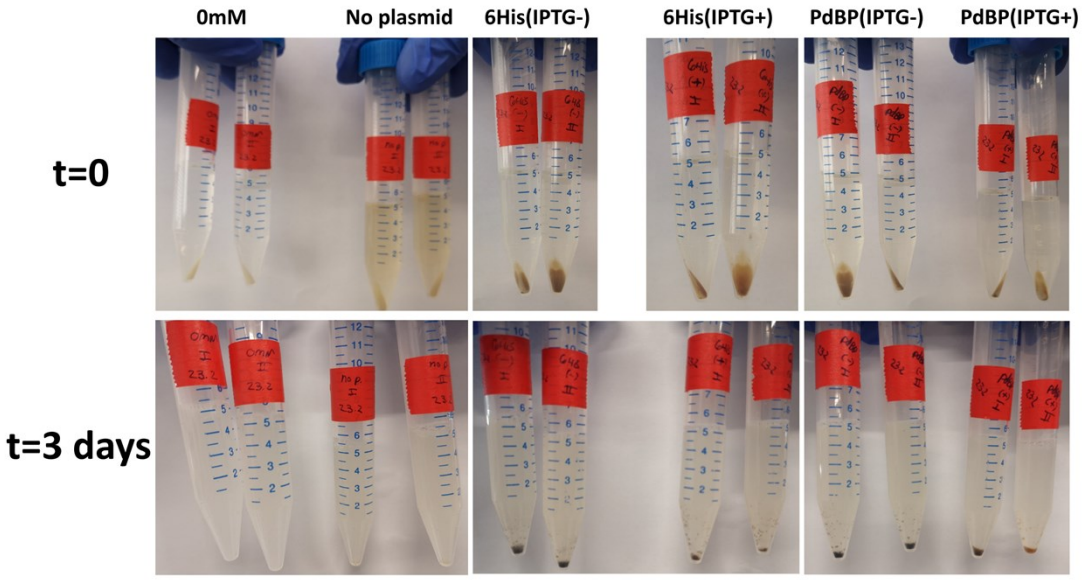

Fig. S6 *in vivo* synthesis of Pd-NPs. The experiment was conducted in duplicates and included samples without $PdCl_2$ (0mM) containing only the bacteria, without expression of SP1 (No plasmid), basal expression of 6His-SP1 and PdBP-SP1 [6His(IPTG-) and PdBP(IPTG-), respectively] and overexpression of 6His-SP1 and PdBP-SP1 [6His(IPTG+) and PdBP(IPTG+), respectively]. Samples are shown immediately after overnight incubation with 2mM $PdCl_2$ (t=0) and 3 days after washing excess of Pd ions (t=3 days).

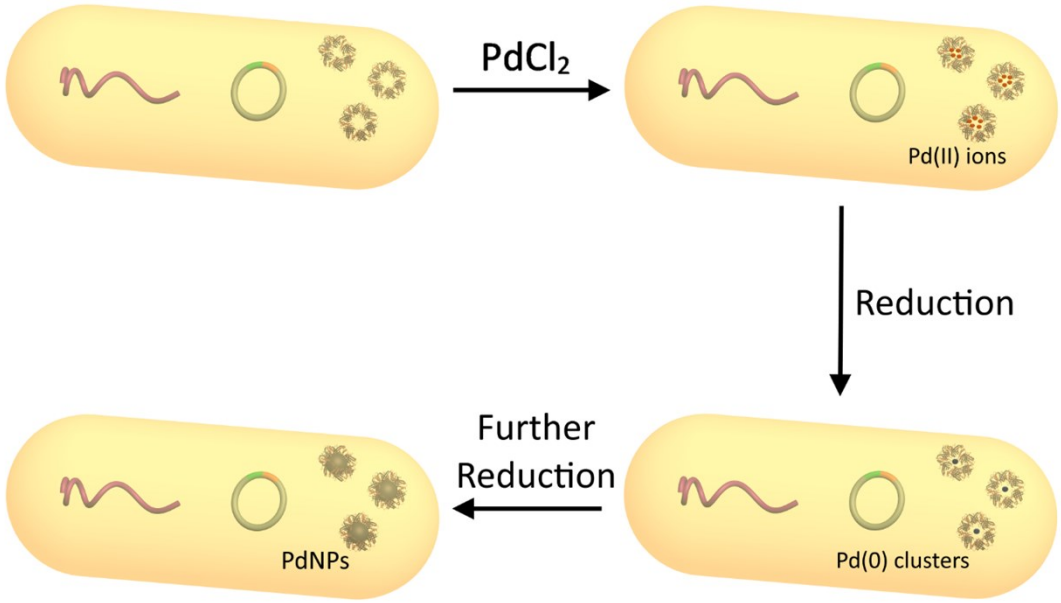

Fig. S7 Proposed mechanism for PdNPs synthesis in 6His-SP1 expressing bacteria. After the addition of palladium salt to the bacterial medium, Pd(II) ions penetrate the cell and accumulate in the cavity of the overexpressed 6His-SP1/ PdBP-SP1 due to its high affinity to Pd ions. A mild reduction by cell intermediates (e.g. NADH, $FADH_2$) can allow the formation of small Pd(0) clusters which can further grow into PdNPs.

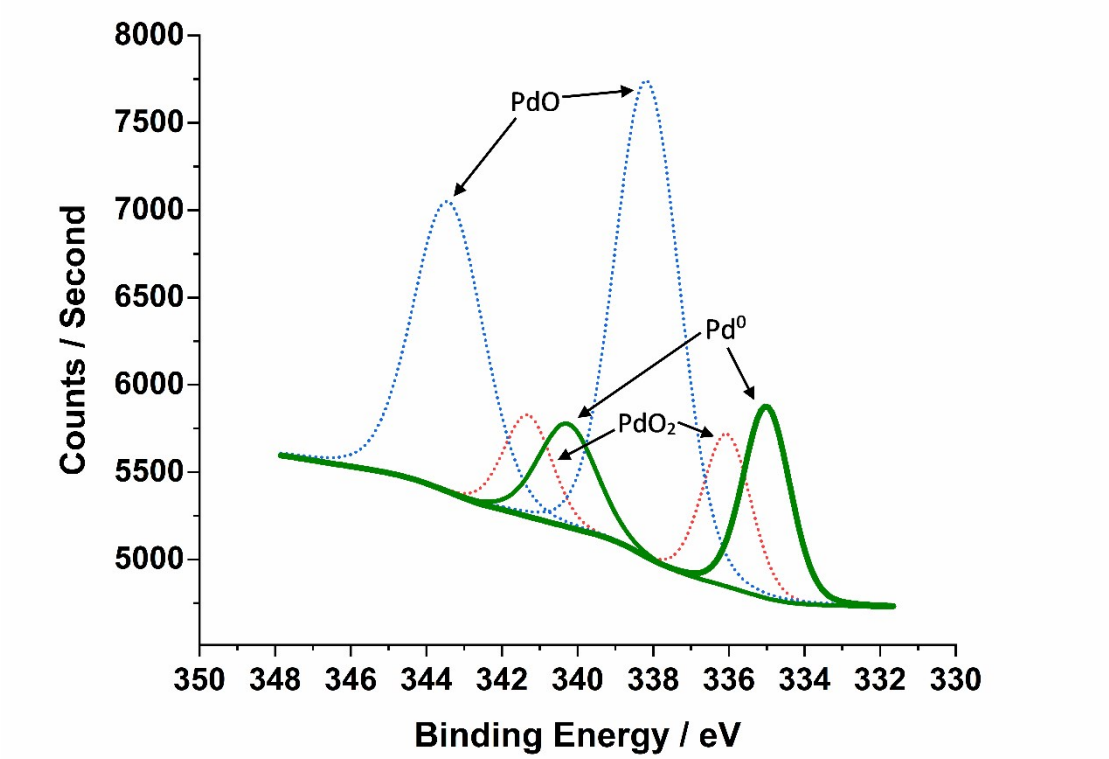

Fig. S8 High resolution XPS spectra of the Pd 3d line of freeze dried *E.coli* BL21 6His-SP1 (IPTG+) cells after PdNPs synthesis. Pd^0, PdO and PdO_2 particles are shown in solid green, dashed blue and dashed red curves, respectively.

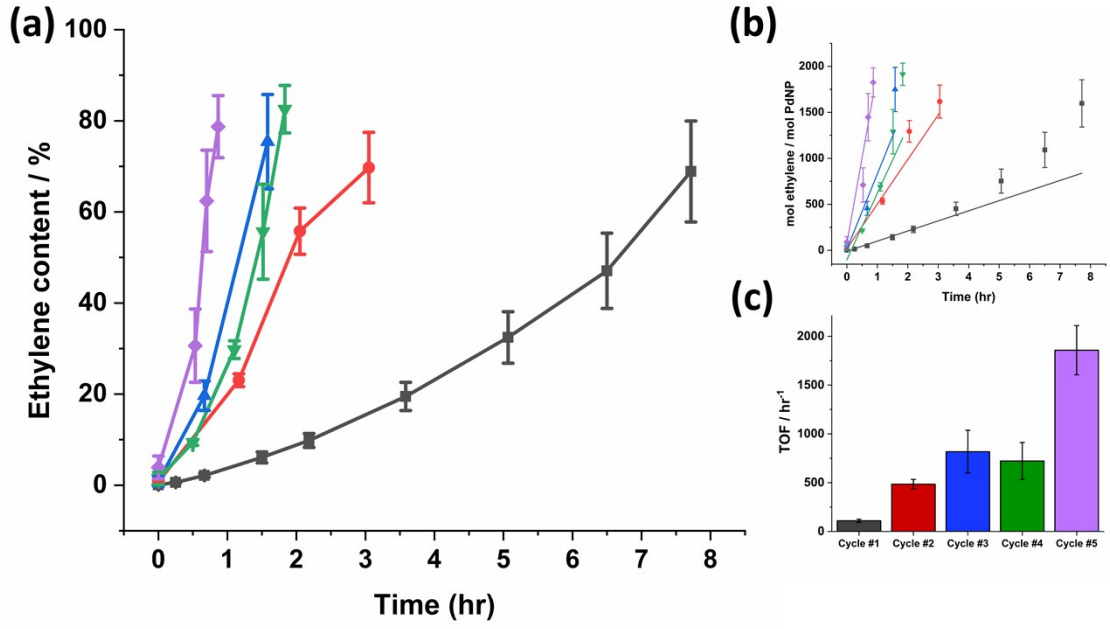

Fig. S9 Reusability test and for hydrogenation catalysis of ethylene production from acetylene feedstock by whole cells expressing 6His-SP1 (IPTG+) after biosynthesis of Pd-NPs. Grey, red, blue, green and purple curves represent cycles number 1,2,3,4 and 5 respectively. (a) Ethylene content over time. (b) and (c) TOF data and TOF comparison for the five cycles, respectively.

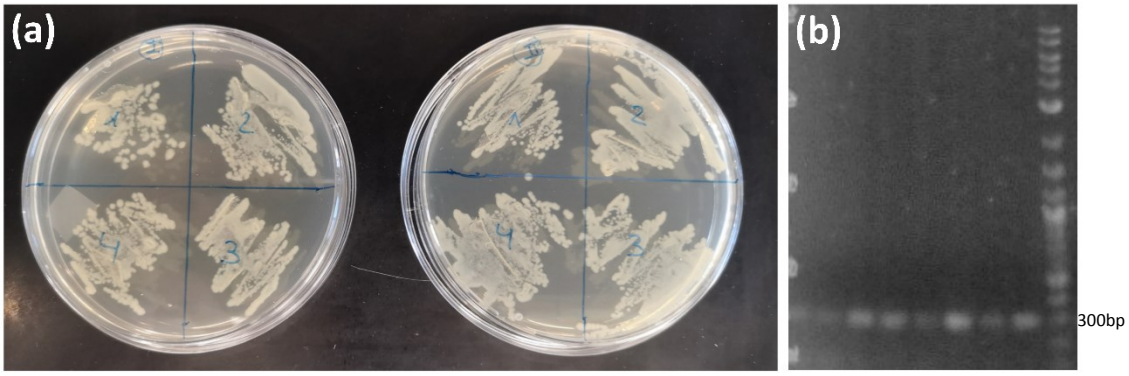

Fig. S10 Viability of *E.coli* BL21 6His-SP1 (IPTG+) cells after reusability test. (a) LB-agar plates spread with cells after the fifth catalytic cycle (conducted in duplicates). (b) 1% agarose gel for colony PCR reactions showing amplification of 6His-SP1 gene for all samples. Right lane – marker. The observed bands are corresponded to ~350bp as expected.

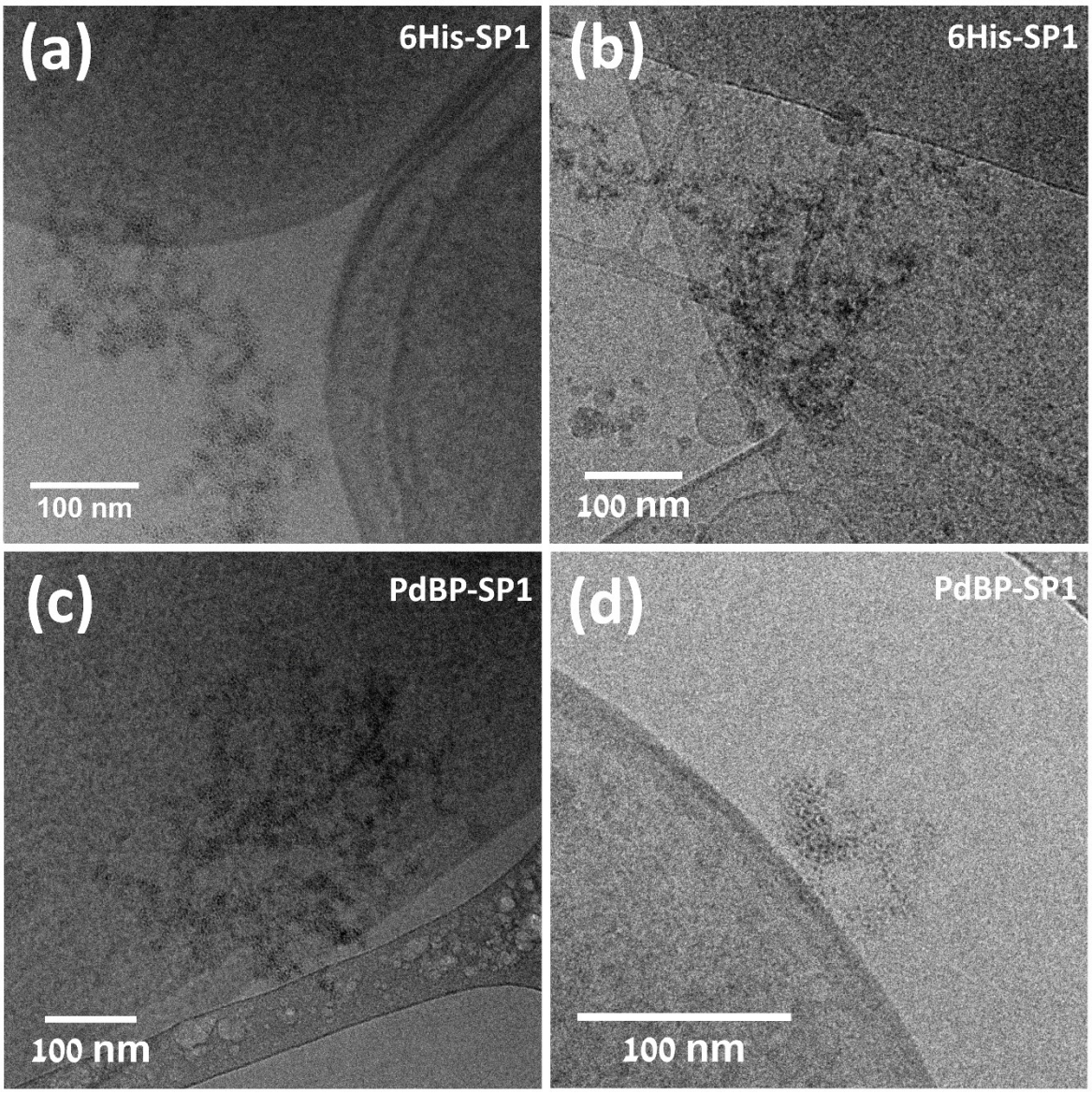

Fig. S11 Cryo-TEM representative images of *E.coli* BL21 cell with basal expression of 6His-SP1 [(a) and (b)] and PdBP-SP1 [(c) and (d)] after 3 days of incubation.

References

1. Arblaster, J. W. Crystallographic Properties of Palladium - Assessment of properties from absolute zero to the melting point, *Platinum Metals Rev* 56, 144-213 (2012).
2. Ausubel, F.M, Brent, R., Kingston, R.E. & Moore, D.D. *Short protocols in molecular biology: a compendium of methods from current protocols in molecular biology*. (Greene Publishing Associates, New York., 1987).