Supporting Information

Coupling plasmonic catalysis and nanocrystals formation through cyclic regeneration of NADH

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Table of Content

1. Materials and Methods

1.1 Chemicals:

Tetrachloroauric acid ($HAuCl_4$, ≥ 99%), hexadecyltrimethylammonium bromide (CTAB, ≥ 99%), sodium borohydride ($NaBH_4$), silver nitrate ($AgNO_3$, ≥ 99%), hydrochloric acid (HCl, 37%), L-ascorbic acid (AA, ≥ 99%) and potassium tetrachloropalladate (II) (K_2PdCl_4, 99.99%), sodium triphosphate pentabasic (>98%), Monobasic potassium phosphate (ACS reagent), sodium hydroxide (>98%), sodium formate (ACS reagent), Polyvinylpyrrolidone (8k), β-Nicotinamide adenine dinucleotide hydrate (98%), β-Nicotinamide adenine dinucleotide, reduced dipotassium salt (NADH) (>95%) were purchased from Merck. Agarose beads were purchased from Bio-Works. All chemicals were used without further purification. Milli-Q water (resistivity 18.2 MΩ·cm at 25 °C) was used in all experiments.

1.2 Instrumentation:

Transmission electron microscopy (TEM) images were collected with a JEOL JEM-1400PLUS, operating at 120 kV. High resolution HAADF-STEM images were acquired using an aberration corrected cubed FEI-Titan 50-80 electron microscope operated at 300 kV. Scanning electron microscopy (SEM) was measured with a dual beam FIB - FEI Helios 450S microscope with electron column resolution of 0.8 nm at 20 kV. 1H NMR spectra of the various samples were recorded in D_2O on an AVANCE III Bruker 500 NMR spectrometer. Optical extinction spectra were recorded using a Cary 5000 UV/Vis/NIR spectrophotometer while emission spectra were acquired using Cary Eclipse. Online UV-vis-NIR characterization was performed with MAYA2000PRO spectrophotometer (Ocean Optics), equipped with DT-MINI-2-63 light source and 2 optical fiber (QP50-2-VIS-NIP: core diameter, 50 μm; length, 2 m) and SMA-Z-10-μvol Teflon flow cell with 10 mm optical path. X-ray photoelectron spectroscopy (XPS) measurements were performed in a SPECS Sage HR 100 spectrometer with a non-monochromatic X-ray source (Aluminum Kα line of 1486.6 eV, 300 W). Thermogravimetric analysis was recorded on a TGA Q500 (TA Instruments) under oxygen by equilibrating at 100 °C and using a ramp of 10 °C/min up to 700 °C. Dark field images were acquired using Nikon SMZ1500 inverted stereomicroscope equipped with digital camera (DN100). GC-MS analysis was performed on a 7820A GC system, coupled to Agilent 5975C Series GC/MSD, with Triple-Axis HED-EM. Glass visualizations in Figure 5 have been rendered with Blender 2.9 using Glass 2.0 shader by CG Vertex.

1.3 Synthesis of gold nanorods

Gold nanorods were prepared using Ag-assisted seeded growth using hydroquinone as reducing agent. Seeds were prepared by reduction of $HAuCl_4$ (5 mL, 0.25 mM) with freshly prepared $NaBH_4$ (0.3 mL, 10 mM) in aqueous CTAB solution (100 mM). After 30 min, an aliquot of seed solution (1 mL) was added to a growth solution containing CTAB (100 mL, 100 mM), $HAuCl_4$ (1 mL, 50 mM), $AgNO_3$ (1 mL, 10 mM), HQ (15 mL, 100 mM). The mixture was left undisturbed at 30 °C for 1 h. The solution was centrifuged twice (8000 rpm, 30 min) and redispersed in CTAB (100 mM) to obtain a final concentration of gold equal to 0.5 mM.

1.4 Pd-coating of gold nanorods

Palladium overgrowth was carried out in the presence of silver ions. To a dispersion of gold nanorods (10 mL, [Au] = 0.5 mM) in CTAB (100 mM) was added $AgNO_3$ (0.04

mL, 10 mM) and K_2PdCl_4 (0.1 mL, 10 mM) and the mixture left for 5 min at 40 ºC to allow for complexation of the palladium salt with CTAB, followed by addition of ascorbic acid (0.2 mL, 100 mM) maintaining the temperature at 40 ºC for 12 h. Palladium-coated gold nanorods were washed twice by centrifugation (8000 rpm, 30 min) and redispersed in water to obtain a final concentration of gold equal to 1 mM.

1.5 AuPd/agarose photocatalyst

A solution of AuPd nanorods (3 mL, 0.001 M in terms of atomic gold) in CTAB (~cmc) was added dropwise to the suspension containing agarose beads (2.5 mL, 0.8 g). The solution was left under stirring 10 minutes until the supernatant became transparent indicating immobilization of the nanoparticles on the agarose beads. The solution was centrifuged twice (1000 rpm, 10 min) and redispersed in water (5 mL). The suspension was stored at room temperature in water.

1.6 Light process in batch

AuPd/agarose composite (0.8 g) was redispersed in a solution (5 mL) containing phosphate buffer (pH 8), sodium formate (1 M), NAD^+ (1 mM) and STTP (50 mM). The mixture was transferred to glass vial (22 mL), sealed with septum. After degassing the mixture with Ar, the photocatalyst was allowed to sediment to form homogeneous film on the bottom of the vial (see **Figure 4a**). The vial was irradiated from the bottom with Fiber-Lite MI-150 light source (Dolan-Jenner Industries), with light power density of 200 mW/cm^2 (400−1200 nm) for 2 hours at 40 ºC. Every 30 minutes, an aliquot was placed in 1 mm quartz cuvette for determination of the total concentration of NADH by measuring absorbance at 340 nm.

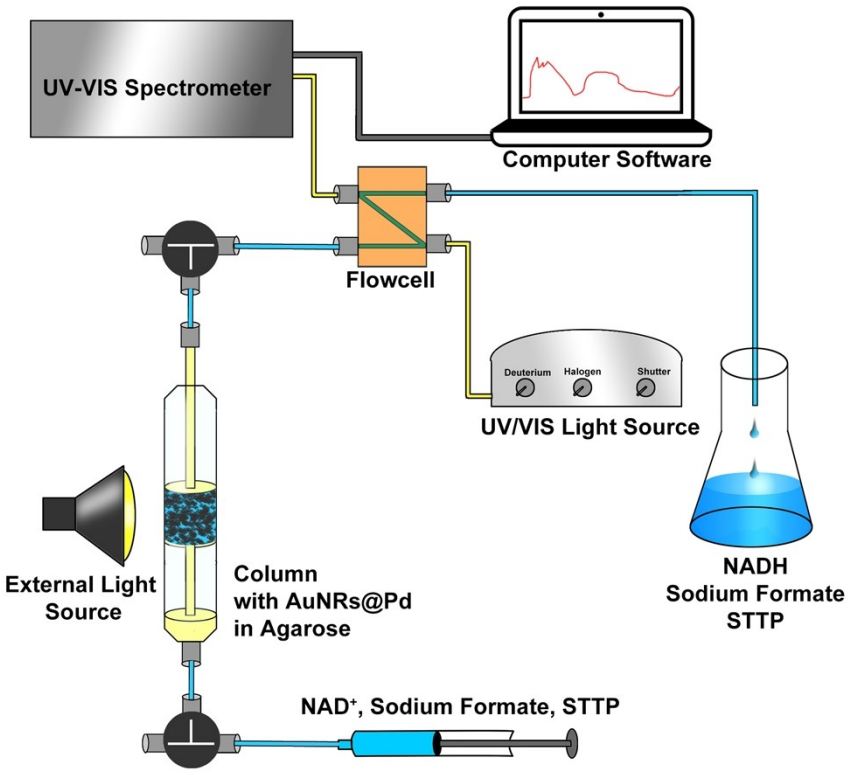
1.7 Dark process (effect of NAD/NADH ratio)

Solutions containing phosphate buffer (pH 8), sodium formate (1 M), STTP (0.05 mM) and different molar ratios of NAD^+ / NADH = 0, 0.25, 0.5, 0.75, 1 ([NAD] = 0.001 M), were added to a solution of $HAuCl_4$ (0.05 M) to give final [Au]=0.00025M. The solutions were left undisturbed for at least 24 hours at room temperature.

1.7 Light process in flow (NADH regeneration)

1.7.1 Design of flow photoreactor aided by real-time UV-vis-NIR

Scheme S1 shows the flow photoreactor used to regenerate NADH. The central component is the flow column (Omnifit) made out of borosilicate glass. The loading of the column with AuPd/agarose photocatalyst is described in **section 1.7.2**. The inlet and outlet of the column were connected to T-valves, both connected with Teflon tubes (ID= 0.7 mm). The column was irradiated with visible halogen light Fiber-Lite MI-150 light source (Dolan-Jenner Industries, power density ranging from 150 to 300 mW/cm^2, 400−1200 nm. The column was cooled with compressed air to keep the temperature inside the column at 33 ºC. The evolution of NADH was monitored in real-time by using Teflon Z-flow cuvette, (Ocean Optics) connected to visible light source (Ocean Optics) and spectrophotometer (MayaPro2000). The spectrophotometer was controlled by OceanView software (Ocean Optics). The inlet syringe (20 mL), containing photocatalytic mixture, was mounted on high precision syringe pump (Alladin1000 WP)



Scheme 1. General scheme of the experimental design of the regeneration of the NADH in flow conditions using real-time analytics.

1.7.2 Loading flow column with AuPd/agarose composite

In all flow experiments we used Omnifit column of 0.342 cm of inner diameter. To fill the column with composite, the column was positioned vertically with connected tubes at the bottom allowing thus the drainage of water. First the column was filled with water and allowed to drain the water to half of the column length, then a suspension of AuPd/agarose was slowly poured from the top using glass Pasteur pipette. The AuPd/agarose were allowed to slowly accommodate on the bottom of the column until reaching 3.5 cm of bed length (total volume = 1.23 mL). Then water (5 mL) was poured through the column and it was closed from the top.

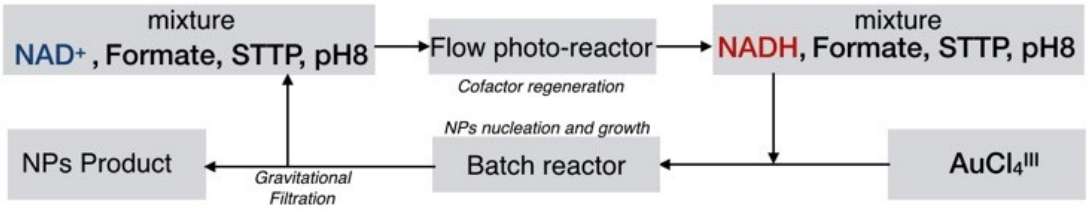
1.7.3 NADH regeneration in flow reactor

In typical experiment, a 20 mL plastic syringe was filled with catalytic mixture containing sodium formate (1M), NAD^+ (0.001 M), STTP (0.05 M) in phosphate buffer (pH8, 0.05 M). For the preparation of each stock solution (sodium formate, NAD^+, STTP) phosphate buffer was used as a solvent. The syringe was connected to the T-valve on the inlet to the column. The optimal flow was set to 0.041 mL/min (residence time = 30 min).

1.8 Cyclic light and dark process (Synthesis of small nanoparticles)

Scheme 2 shows step-by-step procedure of the cylic regeneration of NADH. In typical experiment, a 20 mL plastic syringe was filled in with catalytic mixture containing sodium formate (1 M), NAD^+ (0.001 M), STTP (0.05 M) at pH8. The solution was pumped through photoreactor at flow rate 0.041 mL/min (residence time = 30 min) and

light irradiation 300 mW/cm^2 (400−1200 nm). Once the solution was collected in vial, the concentration of NADH was estimated by UV-Vis (Absorbance 340 nm, molar absorption coefficient 6220 M^{-1} cm^{-1}). Then, a desired amount of $AuCl_4^{III}$ (0.05 M) was added to the solution to reach molar ratio NADH/ $AuCl_4^{III}$ = 4. The solution was left undisturbed for 24 hours, followed by UV-Vis measurements to assess the complete reduction of gold. Next, the solution was filtered through centrifugal filtration in Amicon tubes (3 kDa) at 10000 rpm for 20 minutes. The collected permeate solution contained gold-free solution that was used in the subsequent cycle of light reaction.



Scheme 2. General scheme of cyclic light and dark process.

2. Kinetics of gold nucleation and NADH oxidation

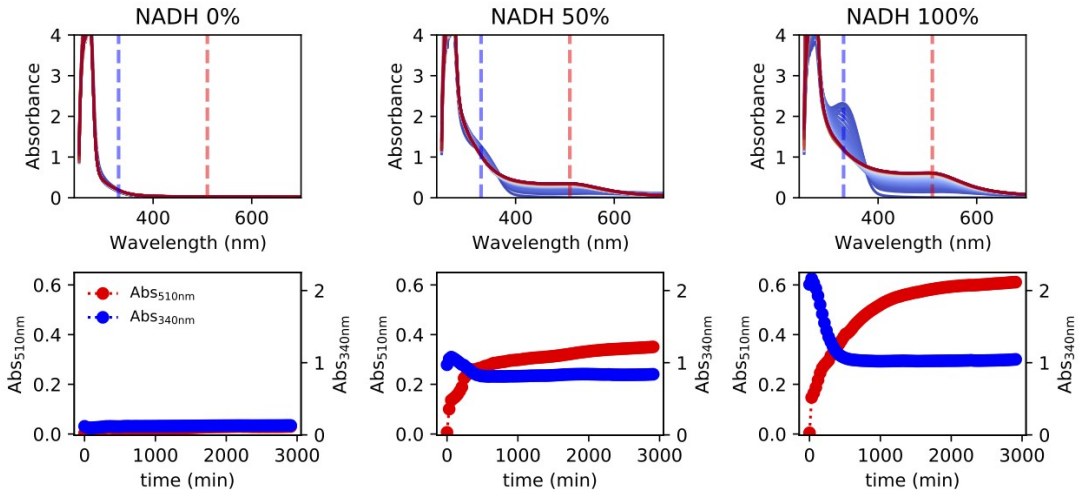


Figure S1. The effect of NAD+/NADH molar ratio on the gold reduction in the dark. (upper panel) UV-Vis spectra taken at the interval of 30 minutes of the solutions containing NAD^+/NADH at different molar ratios (1:0, 1:1 and 0:1). The vertical dashed lines correspond to the wavelengths of NADH (340 nm) and metallic gold (510 nm), which were tracked in the lower panel. (lower panel) The time-dependent change of the absorbance at 340 nm (blue) and 510 nm (red), showing simultaneous oxidation of NADH (a decrease of the absorbance at 340 nm) and reduction of metallic god (an increase of the absorbance at 510 nm). These data confirm that NAD^+ alone is unable to induce the nucleation of gold nanoparticles and NADH is the sole reducing agent. Experimental conditions: $[Au^{III}]$ = 0.25 mM, [NAD] = 1mM, [Sodium Formate] = 1M, pH=8

3. Z-potential of Au NPs obtained in dark reaction

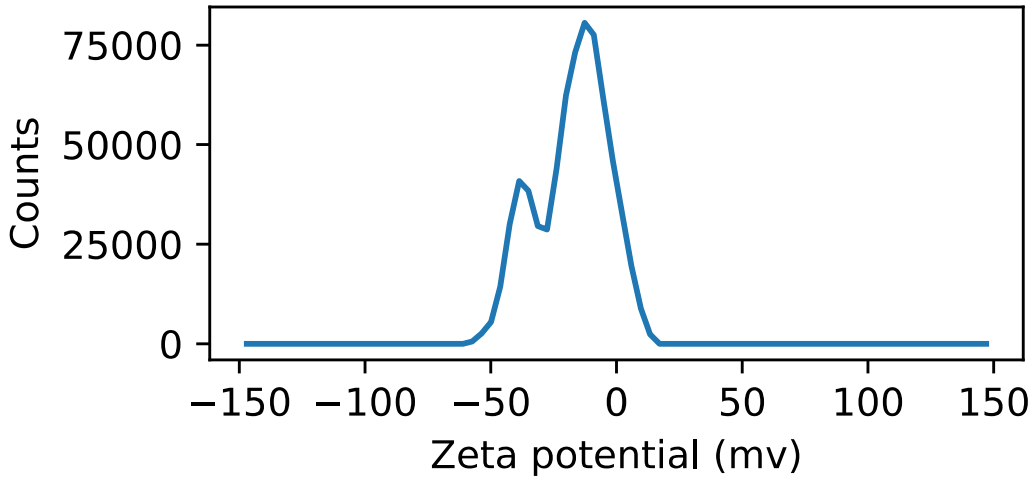


Figure S2. Z-potential of gold nanoparticles prepared in the presence of 100% NADH (0.001 M), STTP (0.05 M), sodium formate (1M) in pH8.

4. ^{1}H NMR of NAD(H) upon gold reduction

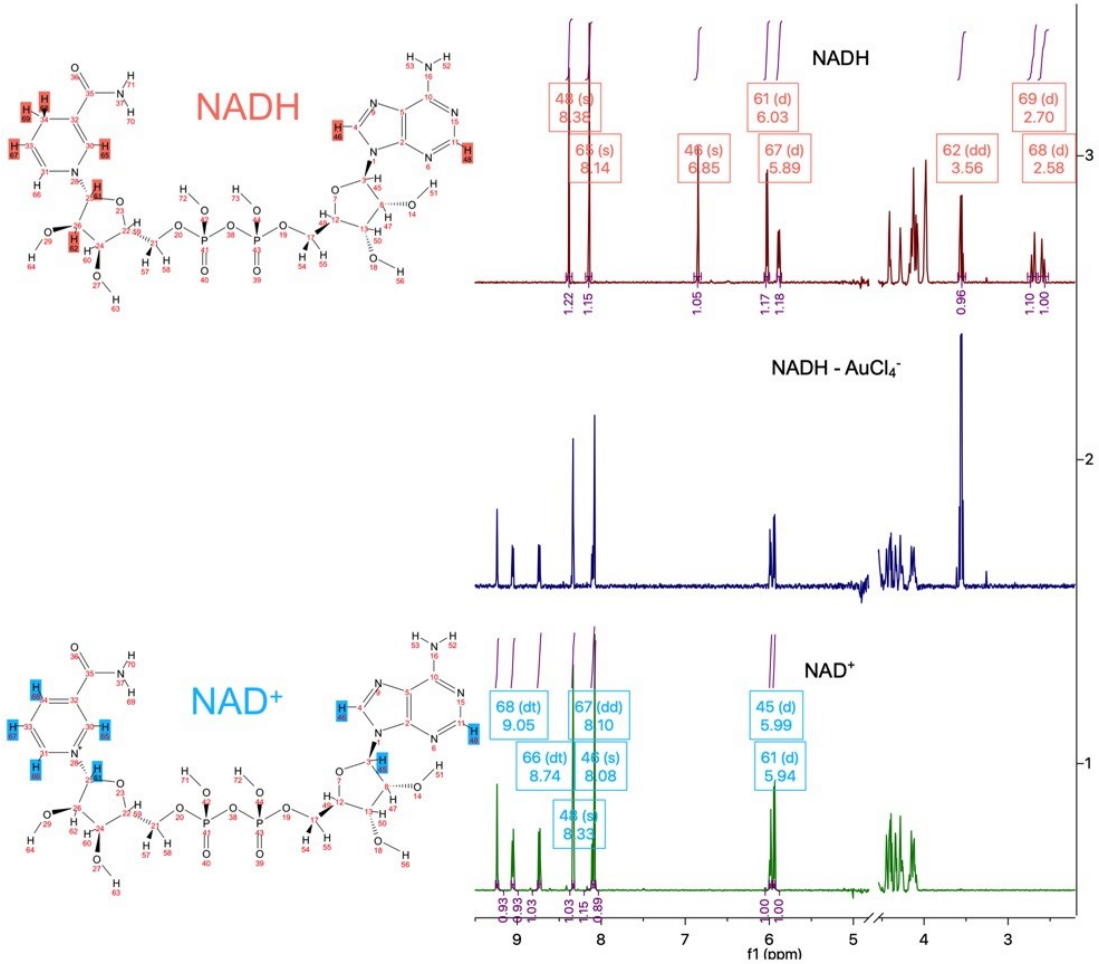


Figure S3. ^{1}H NMR analysis of NAD^{+} and NADH in the presence of $AuCl_4^-$. Upper panel: H-NMR spectrum of commercial NADH. Lower panel: ^{1}H NMR spectrum of commercial NAD^{+}. Middle panel: ^{1}H NMR spectrum of mixture containing NADH and gold precursor at the molar ratio equal to 1:4. The spectrum was acquired 2 hours after the both components were mixed.

5. Effect of conventional stabilizers on light/dark reactions

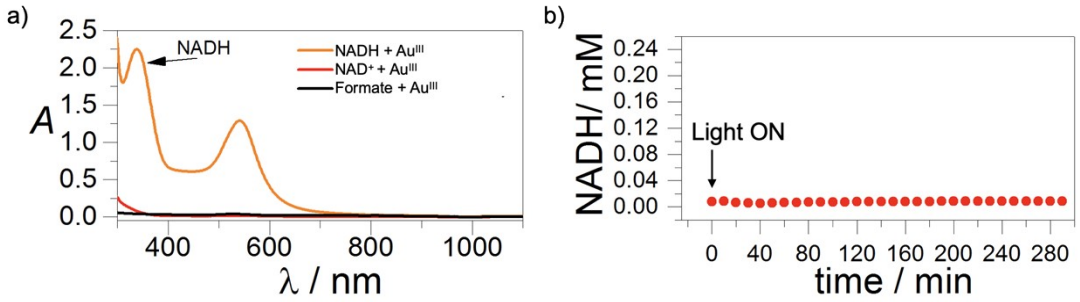


Figure S4. Effect of CTAB as stabilizer on dark (a) and light (b) reactions. a) UV-Vis-NIR spectra of solutions containing gold precursor in the presence of CTAB and sodium formate (1M) at pH8, containing commercial NADH (1 mM), NAD^+ (1 mM) or absence of cofactor. The formation of gold nanoparticles (LSPR at 520 nm) is detected when NADH is used as reducing agent. No gold reduction is observed when NAD+ or sodium formate is used. b) Photocatalytic regeneration of NADH in flow conditions in the presence of CTAB as stabilizer. No NADH is detected suggesting that CTAB by strongly adsorbing to the metal surface via Br^- inhibits ligth process. Experimental conditions in flow: sodium formate (1 M), NAD^+ (1 mM), CTAB (50 mM), pH 8, flow rate: 17 µL/min, Residence time: 90 min, Light power density: 150 mW/cm^2.

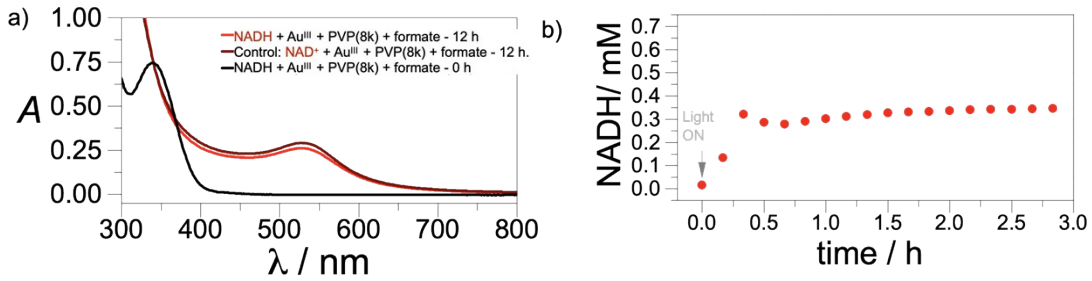


Figure S5. Effect of PVP as stabilizer on dark (a) and light (b) reaction. a) UV-Vis-NIR spectra of solutions containing gold precursor in the presence of PVP (8k, 0.001 M) and sodium formate (1M). Regardless the use NADH or NAD^+, the nucleation of gold nanoparticles is detected by the emergence of LSPR at 530 nm. These results suggest that PVP present in the solution can reduce NAD^+ to NADH non-selectively. b) Light process in flow in the presence of PVP (8k) showing reduction of NADH. These results show that PVP is not a suitable stabilizing agent since it interferes with the photoregeneration of NADH. Experimental conditions in flow: sodium formate (1 M), NAD^+ (1 mM), PVP (8k, 0.001 M), pH 8, flow rate: 17 µL/min, Residence time: 90 min, Light power density: 150 mW/cm^2.

6. HRTEM characterization of AuPd nanorods

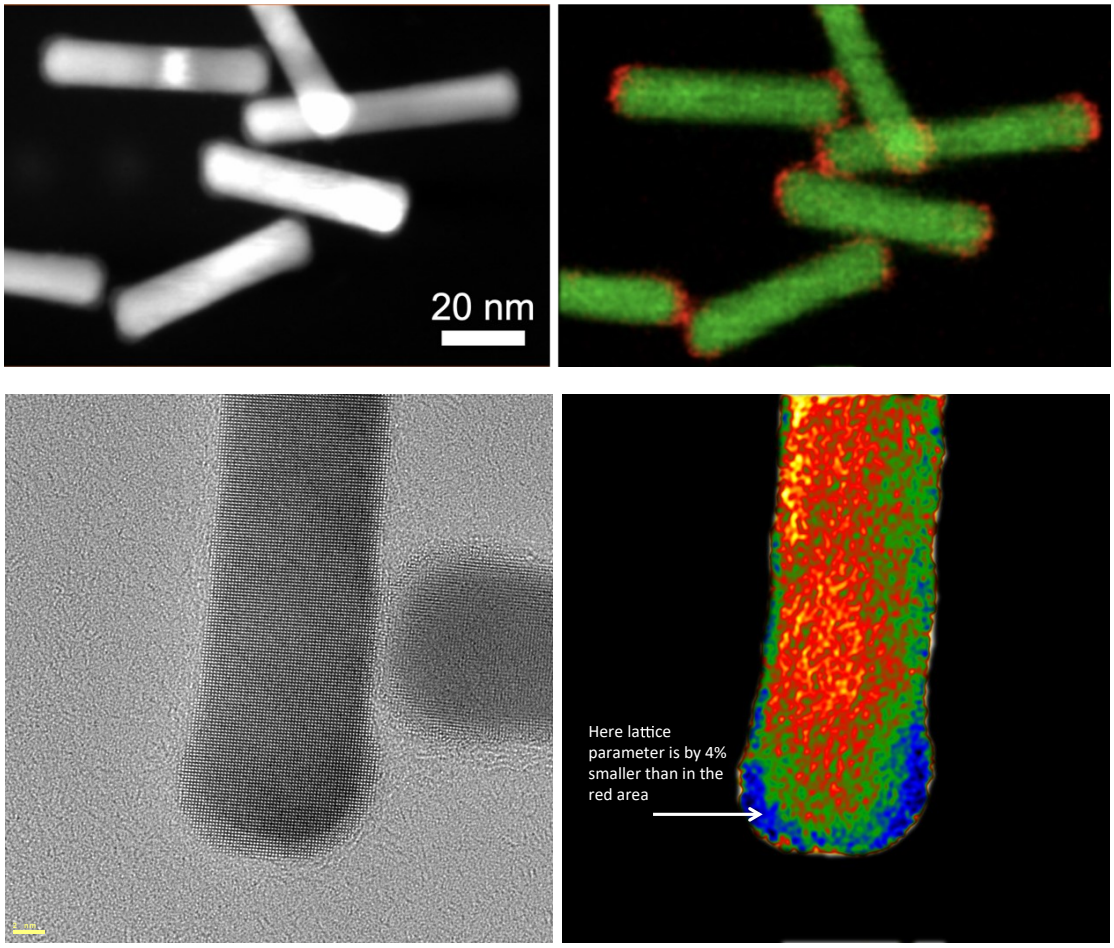


Figure S6. Upper panel: Elemental analysis of the Pd-coated gold nanorods showing the preferential distribution of the metallic palladium on the tips. HRTEM and lattice strain analysis of Pd tip-coated gold nanorods, showing the difference in lattice parameters.

7. Thermogravimetric analysis

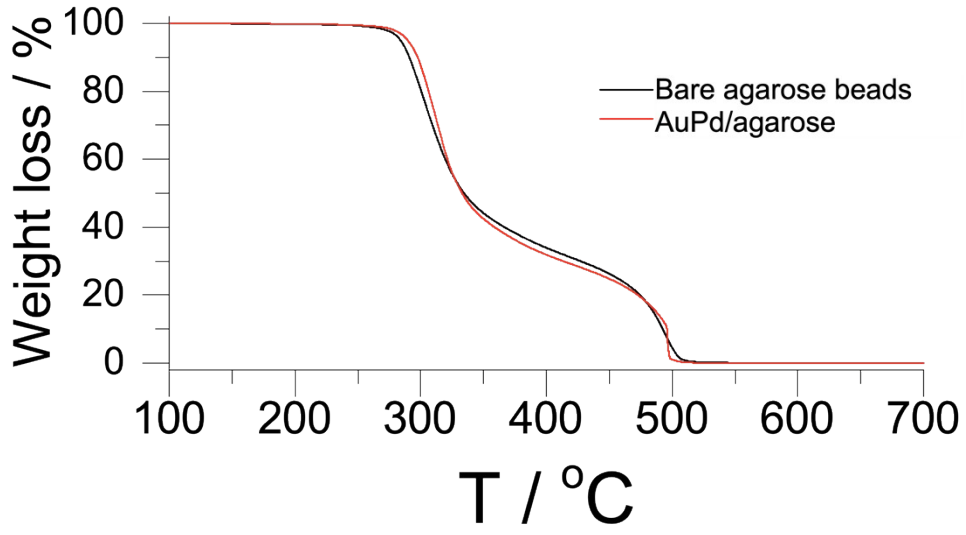


Figure S7. Thermograms of bare and Au/Pd nanorod-loaded agarose beads.

8. Dark Field characterization of AuPd/agarose

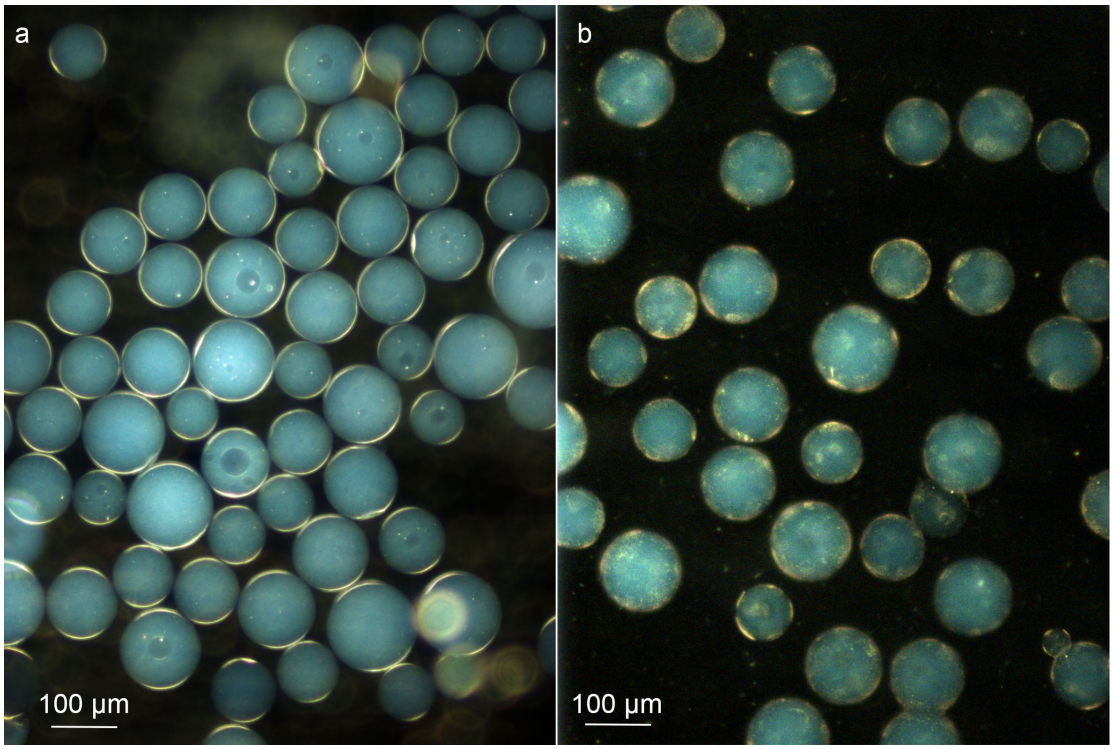


Figure S8. Dark field images analysis of bare beads (left) and AuPd/agarose (right). Bright patches on the beads surface suggest the presence of metallic gold.

9. SEM characterization of AuPd/agarose

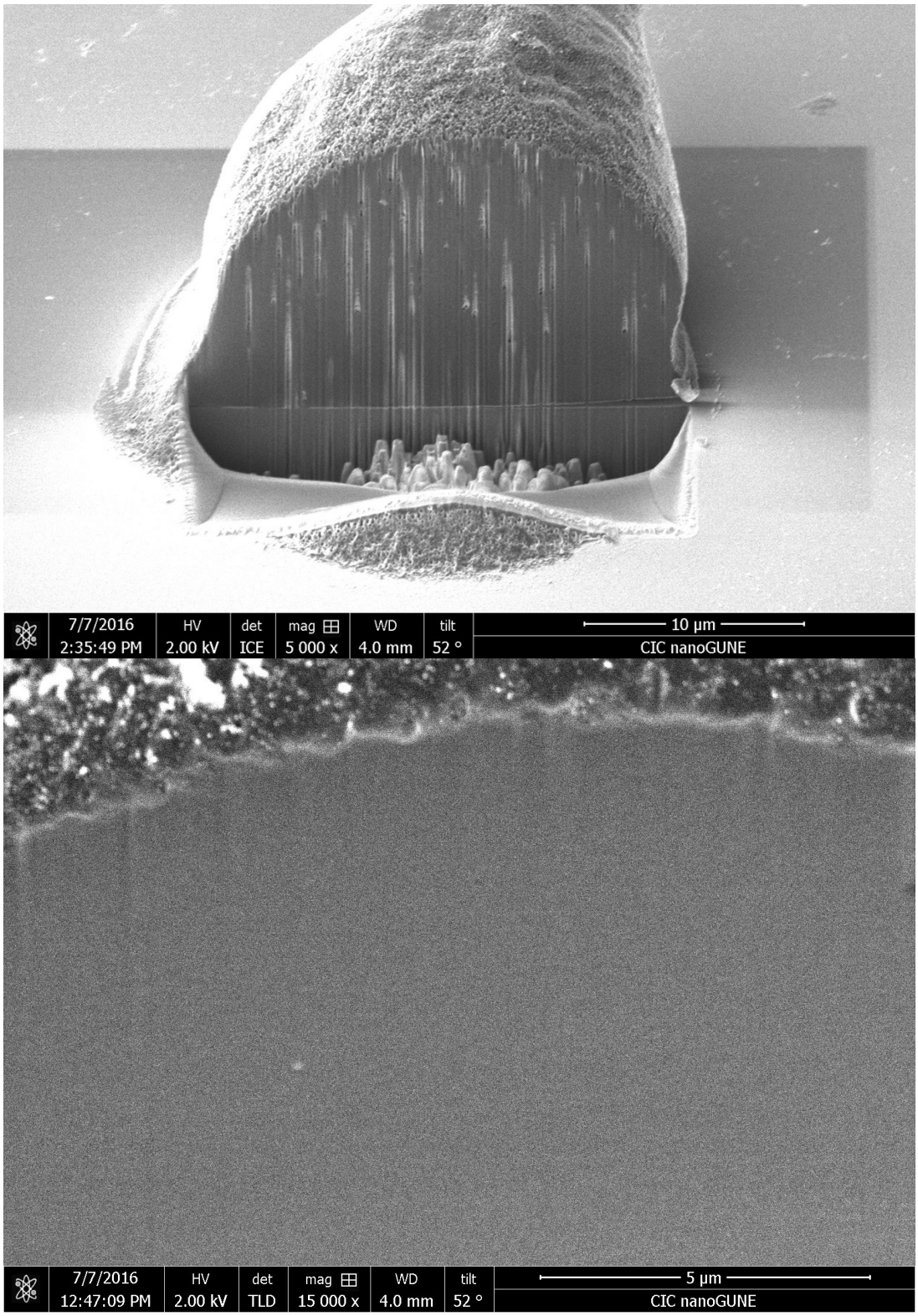


Figure S9. SEM image of individual bead after cutting with focus ion beam.

10. XPS analysis of AuPd/agarose

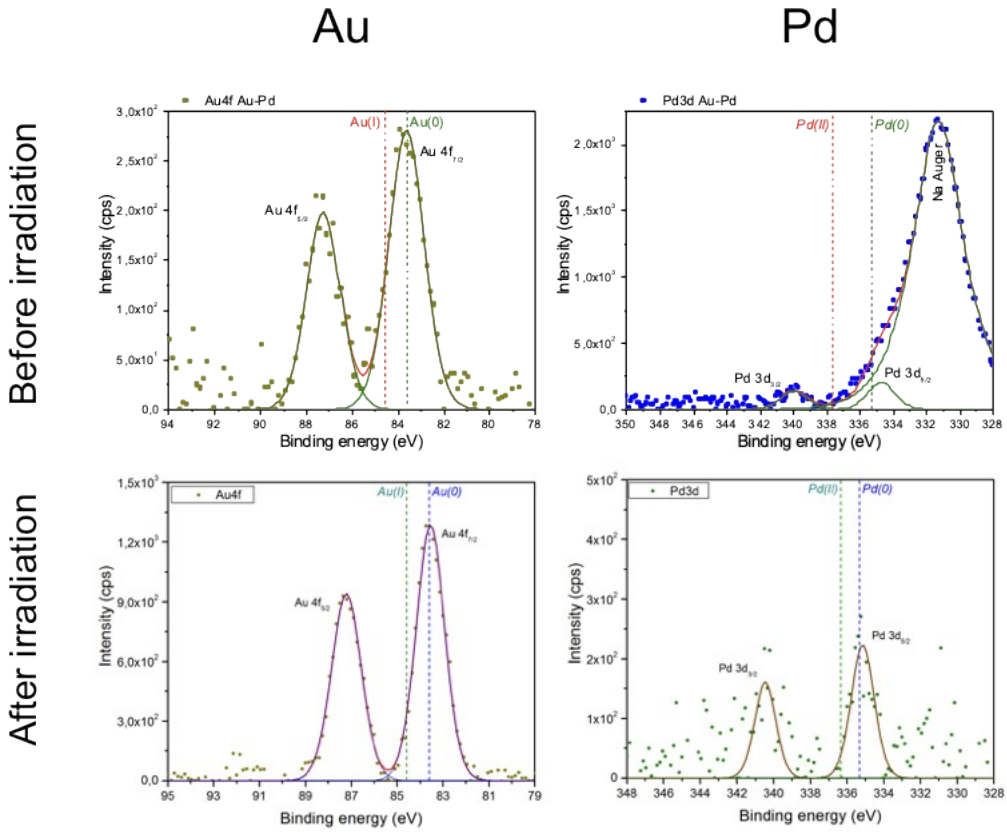


Figure S10. XPS analysis of the AuPd nanorods immoblized on the agarose beads before and after photocatalytic NADH regeneration in flow conditions.

11. Control experiments

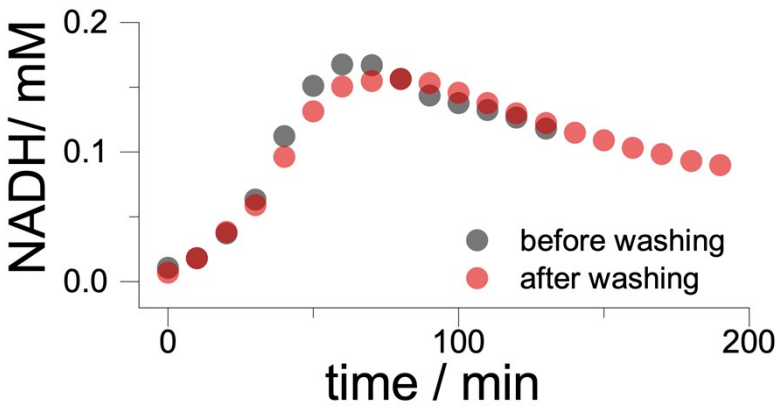


Figure S11. Control experiments showing that the rate of NADH photoreduction (run #1, grey) decreases after reaching the residence time (60 min). The initial rate can be recovered after removal of the AuPd/agarose from the column and its washing by centrifugation. During the second photocatalytic (red), the initial rate of NADH photoregeneration follows the rate of the first process. Experimental conditions: sodium formate (1 M), NAD^+ (1 mM), STTP (50 mM), pH 8, flow rate: 17 µL/min, Residence time: 90 min, Light power density: 150 mW/cm^2.

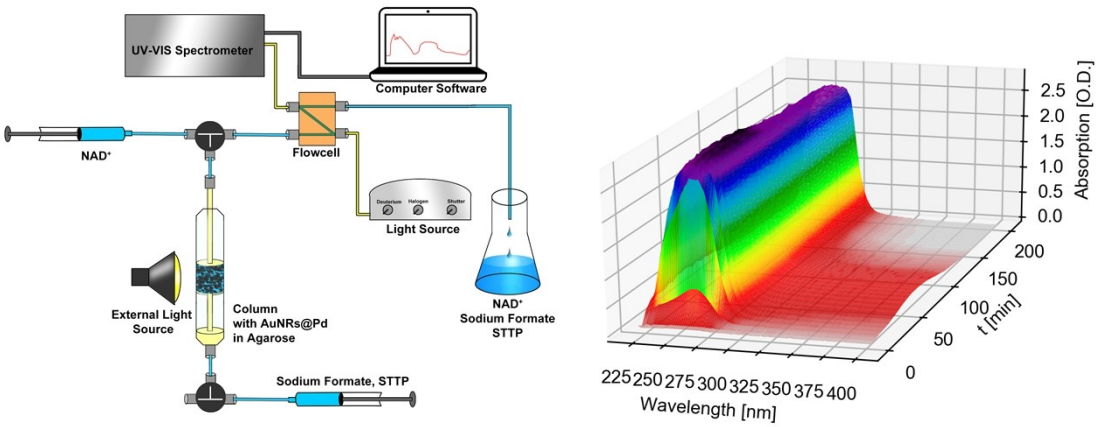


Figure S12. Control experiment confirming simultaneous dehydrogenation of formate and NAD reduction on AuPd surface. The mixture containing sodium formate (1M), STTP (0.05 M) was passed through the column upon light irradiation (200 mW/cm^2). At the column outlet, the irradiated mixture was mixed with NAD^+ (0.001 M) and subjected online UV-Vis measurements. No NADH was observed suggesting that the reduction of NAD^+ to NADH requires AuPd nanoparticles.

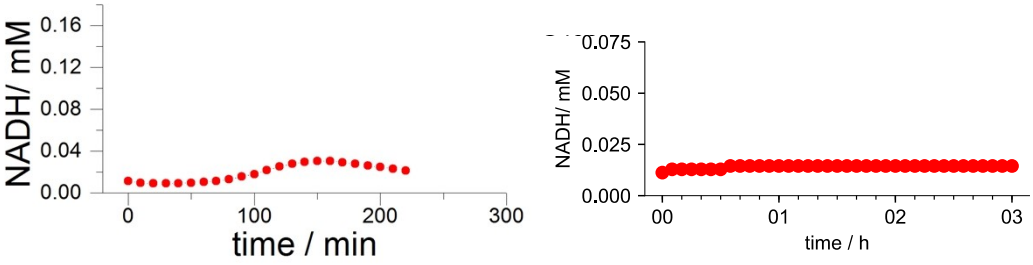


Figure S13. Left: Photoregeneration of NADH in flow using bare gold nanorods (no Pd) immobilized on the agarose beads Approximately, 2% of NADH was produced. Right: Photoregeneration of NADH in flow using agarose only (no AuPd). Experimental conditions: sodium formate (1 M), NAD^+ (0.001 M), STTP (0.05 M), pH 8, flow rate: 17 µL/min, Residence time: 90 min, Light power density: 200 mW/cm^2.

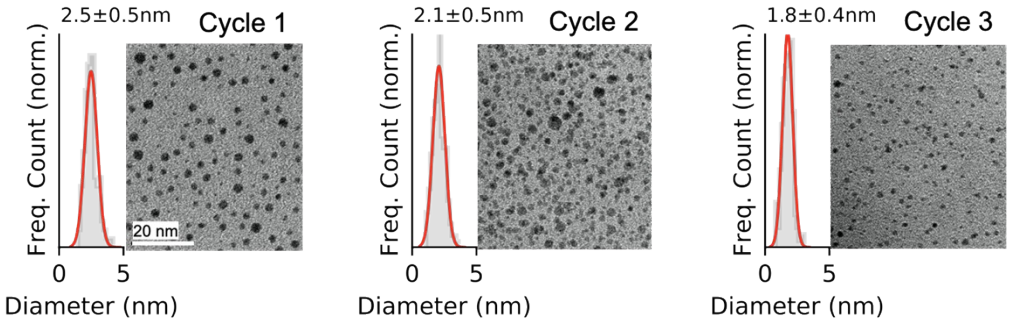


Figure S14. TEM characterization of AuNPs obtained at three consecutive cycles of light and dark reactions.

12. Estimation of turnover number

The calculation of the turnover number of the whole process - the ratio of the number of spherical nanoparticles product to the number of AuPd nanorods photocatalysts – was performed by assuring that (1) During the preparation of AuPd/agarose composite, all CTAB-stabilized AuPd nanoparticles get immobilized in the agarose matrix, (2) During light reaction in flow there is no leaking of AuPd nanorods from agarose beads. By assuring these two requirements, we assumed that the number of AuPd nanorods participating in cyclic photocatalytic reactions remains constant.

Therefore, in a typical light process in flow conditions, each batch of AuPd/agarose composite (see, for example, Figure 4c inset) contained 0.001 M of metallic gold of 45.5±5.0 nm in length and 11.9±1.4 nm in width, giving in total $6x10^{12}$ nanoparticles.

The concentrations and diameters of a new set of spherical gold nanoparticles in each cycle was estimated by measuring the absorbance at 400 nm (after removal of NAD^+ by centrifugal filtration) and analyzing TEM images (Figure S14) giving the following results: cycle 1: [Au] = 0.00010 M, diameter = 2.5±0.5 nm; cycle 2: [Au] = 0.00007 M, diameter = 2.1±0.5 nm and cycle 3: [Au] = 0.00003M, diameter = 1.8±0.4 nm. From

these data the number of spherical gold nanoparticles was estimated for cycle 1, 2 and 3 giving $6x10^{14}$, $8x10^{14}$, $7x10^{14}$, respectively.

Assuming that the number of AuPd nanorods in the flow reactor remained unchanged in each cycle, the ratio of spherical to AuPd nanoparticles gives the following turnover numbers: 100, 133, and 117.