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Cite this: DOI: 10.1039/c0xx00000x

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ARTICLE TYPE

Plasmon-driven photoregeneration of cofactor molecules

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Received (in XXX, XXX) Xth XXXXXXXXXX 20XX, Accepted Xth XXXXXXXXXX 20XX

DOI: 10.1039/b000000x

5 **Platinum-doped gold nanorods are able to regenerate cofactor molecules under visible and infrared light irradiation. Our results suggest promising use of plasmonic particles in biochemical reactions.**

Spurred by outstanding optical properties, metallic nanoparticles gain progressive attention as photocatalysts.^{1–3} Large absorption cross-sections and chemical stability allow metal nanoparticles to efficiently harvest light over UV-Vis-NIR spectral range and use it to catalyse reduction/oxidation reactions.^{4,5} These photochemical processes are mediated by plasmon resonances, which lead to production of heat (phonon), energetic carriers (hot electrons and hot holes) or strong enhancement of optical near field, which increase the efficiency of chemical reactions. The efficiency of near field enhancement has been reported to be related to the shape of the particles. Gold nanorods (AuNRs) for example, strongly enhances the near optical field on their tips because of longitudinal plasmonic resonance.⁶ The chemical processes taking place on the particles surface require often the presence of a co-catalyst (e.g. Co, Pt, Pd), which ensures charge heterogeneity on bimetallic surface, and therefore enhance catalytic activity.⁷ Finally, the distance between particles plays also important role in the light harvesting process. Nanoparticles in an aggregated state enhance incoming light intensity because of strong plasmon coupling, thus making the photochemical process more efficient.⁸ All these parameters are very important when designing plasmonic particles for catalytic purposes and, surely, recent advances in colloidal fabrication offer a broad library of nanocrystals with suitable optical properties and chemical composition.

Cofactors are biomolecules that have colossal importance in proper functioning of living matter. For example in the process of photosynthesis cofactor molecules store light energy by their temporal reduction for later use in respiration and oxidation processes. Thus the subtle balance between oxidized and reduced forms is pivotal for correct biological functioning. During the last 30 years a large body of work has been devoted to non-enzymatic regeneration (reduction) of cofactors using electrochemical and photochemical approaches. Nicotinamide adenine dinucleotide (NADH), as a model cofactor, has been regenerated using semiconductors,⁹ organic dyes¹⁰ or polymers¹¹ as photocatalysts. The attempts toward non-enzymatic regeneration of cofactor molecules need further development and here is where plasmonic particles can surely contribute.

Here we show that gold nanorods functionalized with metallic platinum can regenerate NAD⁺ to NADH using visible and IR light. We found that control over the spatial distribution of cocatalyst (Pt) on AuNRs surface and Au-Pt heterojunctions are crucial elements when performing photochemical processes.

As a photocatalyst we chose gold nanorods¹² (length: 60.4±6.9 nm; width: 13.1±1.6 nm) since they efficiently absorb light over a wide spectral range and offer surface-specific reactivity due to their intrinsic shape anisotropy.¹³ The incorporation of metallic platinum to gold nanorods has been shown to promote photochemical processes.⁷ To reduce platinum on the gold surface (AuNRs@Pt) we reproduced a well-established experimental protocol using ascorbic acid as mild reducing agent.¹⁴ As expected, Pt deposition resulted in a redshift of the longitudinal surface plasmon resonance (LSPR), confirming reduction of the Pt domains selectively on the nanorods tips (Fig. 1b).¹⁵ We hypothesize that upon irradiation, strong optical near field enhancement concentrate photons in the proximity of Pt domain located on the tips, increasing the reduction rate of NAD⁺ to NADH (Fig. 1a). The importance of Pt in our system is supported by recent studies on photochemical regeneration of NADH where Pt nanoparticles have been shown to enhance cofactor regeneration.¹⁶

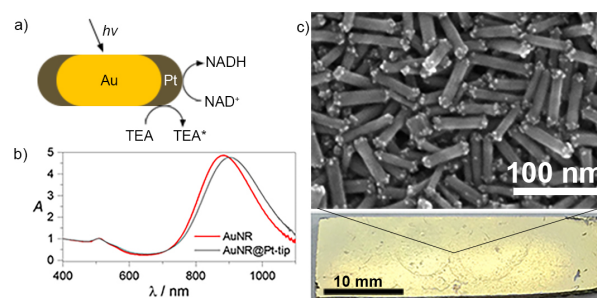


Fig. 1 Gold nanoparticles used in the photocatalytic regeneration of cofactor molecules. (a) scheme illustrating plasmon-assisted photoreduction, (b) UV-Vis-NIR spectra of solutions containing AuNRs before (red) and after (grey) Pt reduction on their tips, (c) SEM and digital images of the plasmonic substrate consisting of AuNRs@Pt.

Photocatalytic processes carried out in colloidal phase often lead to aggregation of the particles, decreasing the initial surface area, and thereby suppressing the catalytic activity.¹⁷ Chemical degradation of the stabilizing agent, proton gradient or even pronounced convection of the nanoparticles are the main reasons for such catalytic inhibition.‡ To avoid uncontrolled aggregation during the photocatalytic process, controlled films of AuNR@Pt were prepared. Slow evaporation of the AuNRs@Pt solution on a glass substrate (2 cm²) led to formation of opaque films

consisting of randomly distributed particles, with a homogenous thickness of $\sim 2 \mu\text{m}$ (Fig. 1c and Fig. S2, S3, ESI†). The films can be washed with ethanol to remove excess surfactant and once immersed in water they show no sign of dissolution or NR release. This is a remarkable feature, especially because no crosslinking molecules were used. Despite of the $2 \mu\text{m}$ thickness of the film, we expect that the nanorods located on the top layers will preferentially participate in NADH photoregeneration reactions, as recently shown in theoretical studies.¹⁸

To perform photocatalytic reactions, the plasmonic films were immersed in a mixture (1 mL) containing NAD^+ (1 mM), phosphate buffer (pH=8) and TEA as sacrificial agent.¹¹ The reduction of NAD^+ was initiated under light irradiation (350 – 1100 nm, 100 mW/cm²) at constant temperature 35 °C. The plasmonic substrates could be removed from the solution at any time for spectroscopic validation of the regeneration process. Since NADH exhibits a characteristic absorption band at 340 nm (molar absorption coefficient 6220 M⁻¹cm⁻¹), UV-Vis spectroscopy provides a facile qualitative and quantitative analytic control of the process.

In the first set of experiments we evaluated the photocatalytic performance of four different substrates consisting of (1) bare AuNRs, (2) Pt NPs (3 nm), (3) a physical mixture of bare AuNRs and Pt NPs, and (4) AuNRs@Pt (Fig. 2). We observed that the photoconversion of NADH increases in the above order, suggesting that the heterojunction between Au photocatalyst and Pt cocatalyst is crucial in this reaction. These results are consistent with recent work by Ouyang and co-workers who showed that tight interaction between metallic subunits is fundamental in plasmon-driven photocatalysis.¹⁹ In addition, the importance of the Pt cocatalyst excludes the thermal effect of the plasmonic nanoparticles. Otherwise, one should observe cofactor regeneration on the substrate consisting of bare AuNRs. Apart from spectroscopic evidences of NADH photoregeneration we qualitatively confirmed the presence of NADH by NMR analysis (Fig. S4, ESI†). It is worth mentioning that Pt alone catalyses NADH photoreduction (Fig. 2) which it consisted with recent work on direct photoexcitation of adsorbate-metal bond using Pt NPs as photocatalyst.²⁰ This observation also supports our hypothesis and confirms the importance of plasmonic nanoparticles (AuNRs) that enhance the near electromagnetic field and thereby the efficiency of chemical conversion.

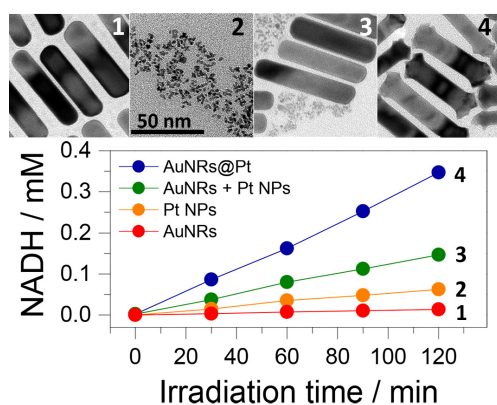


Fig. 2 Effect of Au-Pt heterojunction on photoregeneration of cofactor molecules. Conversion of NAD^+ to NADH on different plasmonic substrates containing (1) bare AuNRs, (2) Pt NPs, (3) physical mixture of bare AuNRs and Pt NPs and (4) AuNRs@Pt.

Next we studied the effect of the size of Pt domains on the regeneration reaction. We prepared plasmonic substrates comprising gold nanorods functionalized with different amounts of Pt on their tips (5, 10 and 15 mol% with respect to the initial Au concentration). We observed that the photoconversion of NADH is 4 times higher for the substrate containing 10 mol% Pt as compared to the substrate containing 5 mol% Pt. Further increase of Pt amount (15 mol%) decreased the amount of reduced NADH (Fig. 3). The excessive amount of Pt can damp the electromagnetic field enhancement around the tips decreasing light absorption, leading to lower rate of NADH photoreduction.

Spatial distribution of the Pt domains on the AuNRs surface also affects the overall catalytic performance. We prepared plasmonic substrates containing AuNRs functionalized with homogeneous Pt shells (10 mol%).¹⁴ Photoregeneration of NADH on the Pt-overcoated nanorods was 3 fold lower as compared to that on tip-coated AuNRs (Fig. 3b). Our observations are in very good agreement with recent results by Majima and co-workers, who showed better hydrogen evolution rate on Pt-tipped AuNRs as compared to Pt-overcoated AuNRs.²¹ The better performance of AuNRs with Pt domains on their tips is due to shape anisotropy of the photocatalyst and more efficient charge separation.

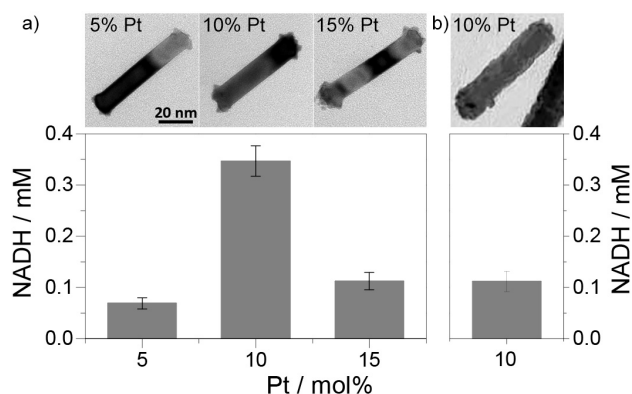


Fig. 3 Effect of Pt domains on cofactor photoregeneration. Concentration of regenerated NADH molecules (2h of irradiation) for different substrates containing AuNRs coated with 5, 10 and 15 mol% of Pt.

The use of gold nanorods provides unique advantages for cofactor regeneration under IR light. We evaluated NADH regeneration at different wavelengths (400 – 1000 nm, for full and individual spectral profiles see Fig. S5, ESI†) using bypass filters (light width ± 10 nm). Indeed, the plasmonic substrates regenerate NADH at an incident wavelength of 1000 nm (Fig. 4). The lowest regeneration rate is observed at 700 nm, which further increases to reach the maximum at 500 nm of incident light. The mismatch between the absorption spectrum of the film and the conversion rate is due to the complex optical properties of the plasmonic films. As recently suggested, plasmonic films of randomly oriented AuNRs strongly scatter light above 500 nm, which is probably related to plasmon coupling effects.¹⁸ We measured diffuse reflectance of films composed of either AuNRs or AuNRs@Pt (Fig. S6, ESI†), which showed that incoming light above 500 nm is reflected while light below 500 nm is absorbed and used in the photochemical process. These experiments therefore provide interesting insights in plasmonic nanochemistry. As commonly accepted, the conversion rate should follow the optical response of individual plasmonic

particles (action spectrum). For the collective system however, especially when containing thousands of nanoparticles with small inter-particle gaps, the relation between the optical response and photo-regeneration is somehow counter-intuitive, and more detailed studies are required to evaluate our observations.

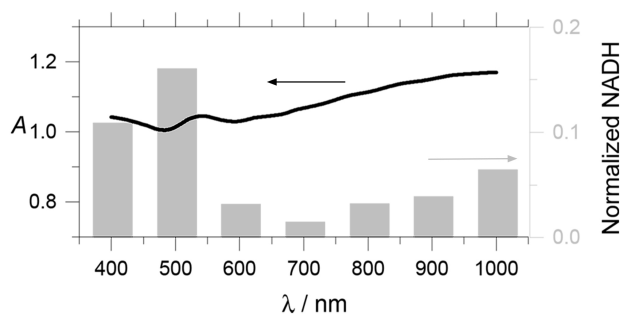


Fig. 4 Wavelength-dependent photoregeneration of NADH on a plasmonic substrate (AuNRs@Pt, 10 mol% Pt, 30 min. irradiation).

Direct plasmonic photocatalysis requires strong binding between adsorbate and nanoparticles surface, which manifests by perturbation of electronic structure.¹ To confirm NAD⁺ interaction with the metal surface we subjected AuNRs@Pt to the NAD⁺ solution (no TEA), observing an LSPR redshift of 4 nm, indicating electron-withdrawing of cofactor molecules, and thus direct electronic interactions (Fig. S7a, ESI[†]). In another control experiment we subjected AuNRs@Pt to pure TEA solution (no NAD⁺), observing a 5 nm LSPR blueshift under light illumination (Fig. S7b, ESI[†]). Such a shift indicates cathodic polarization of the nanorods in the presence of the reducing agent, and therefore suggests the electron transfer from the molecular scavenger to the nanocrystals.²² We found also that the TEA has important effect on the overall process. Progressive decrease of TEA concentration decreases the rate of NAD⁺ reduction (Fig. S7c, ESI[†]), suggesting that the oxidation of sacrificial molecules determines the rate of the cofactor regeneration.

Finally, we ask whether a simple bulk gold film can perform a similar reaction instead of elaborated nanorods with precisely placed Pt domains on the tips? We used a bulk gold film (~200 nm thick), pre-treated with Pt salt and ascorbic acid, for photoregeneration reaction, noticing negligible amount of NADH (Fig. S8, ESI[†]). Although the differences in the surface area of both type of substrate can be important, these results suggest substantial effect of plasmon coupling, which seems to be achievable on rough surfaces with sub-nanometer gaps between the particles.

Conclusions

We demonstrated that gold nanorods doped with small platinum domains can regenerate cofactor molecules under visible and infrared light irradiation. Our results provide an interesting model system for better understanding plasmon-driven processes, thus we prioritize a number of questions for further study. For example, the effect of the aspect ratio of gold nanorods; the effect of plasmon coupling by controllable inter-particle distances; the possible use of water to regenerate cofactor molecules. Of course we expect to increase the efficiency of the process by incorporation of semiconductor subunits on the lateral

parts of the nanorods.¹⁹ From a more general perspective, given that NADH is a highly relevant molecule in biochemistry we anticipate the possibility of creating bioinorganic systems capable of modulating important biochemical processes using light as energy input.

Funding is acknowledged from Spanish Ministry of Education (MAT2013-49375-EXP). M.G. acknowledges the receipt of a fellowship from IKERBASQUE, the Basque Foundation for Science. We thank Luis M. Liz-Marzán and Javier Garcia de Abajo for fruitful discussions and Ada Herrero Ruiz for assistance with NMR analysis.

Notes and references

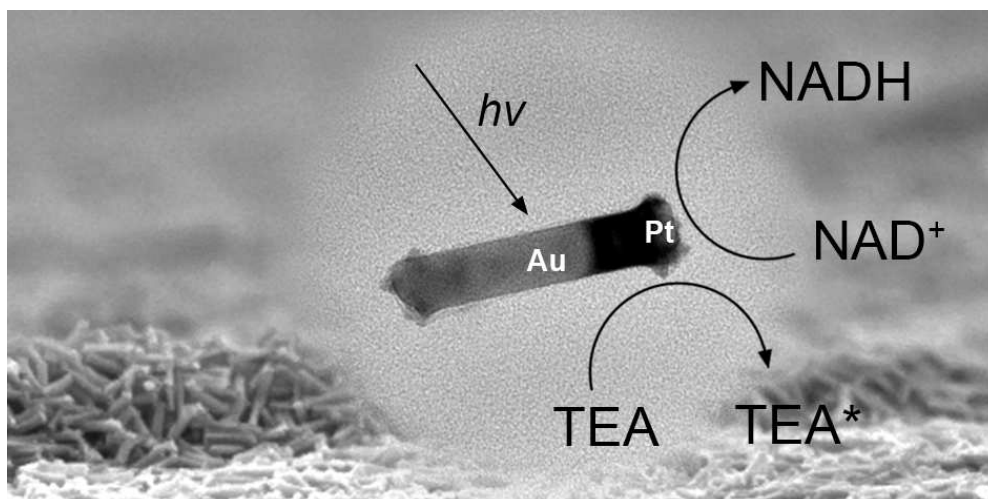
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[†] Electronic Supplementary Information (ESI) available: Methods, SEM images, UV-Vis from control experiments, NMR analysis. See DOI: 10.1039/b000000x/

[‡] In our initial attempt we subjected AuNRs@Pt in colloidal phase for the regeneration of NADH, showing regeneration ~3% accompanied by the particles aggregation (Fig. S1, ESI[†]).

[§] To perform optical characterization we used thinner plasmonic substrates in this set of experiments.

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