

Gold Nanoparticles Surface Plasmon Field Effects on the Proton Pump Process of the Bacteriorhodopsin Photosynthesis

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Gold nanoparticles have three important properties: (1) they absorb and scatter light strongly, a property that is used in sensing, imaging, and medical diagnostic;¹ (2) the absorbed light is rapidly converted into heat which is useful in many photothermal applications, like destroying cancer cells;² and (3) surrounding them when their localized surface plasmon oscillations are excited are plasmonic fields that decay with distance.^{3,4} These surface plasmon fields can affect photochemical processes (surface plasmon photochemistry)⁵ and photophysical processes (surface plasmon enhanced photophysics).⁶ Our group previously reported that plasmonic fields do slow down the retinal photoisomerization in the primary step of bacteriorhodopsin (bR) photosynthesis.⁵ We also found that they can enhance the rate of cooling of hot electrons in semiconductor.⁶ The present contribution is a report on experiment suggesting that while the plasmonic field of gold nanorods (NRs) does not affect the proton deprotonation rate, it speeds up the reprotonation rate of the Schiff base proton pump process in the bacteriorhodopsin photosynthetic system. Qualitative discussion of this observation is given.

BR is a small (26 kDa) transmembrane integral protein found in *Halobacterium salinarium*.⁷ The protein binds a chromophore, a protonated retinal Schiff base, to one of the seven transmembrane helices. Upon photon absorption, the protein and the chromophore undergo a photocycle involving several intermediates where structural rearrangements take place and a proton is transported through the membrane, from the cytoplasmic half-channel to the extracellular half-channel. The resulting electrochemical gradient stimulates the synthesis of ATP through ATP synthase. Among all the intermediates, M_{412} is the most studied of all. During its formation ($L_{550} \rightarrow M_{412}$ process), the Schiff base is deprotonated by Asp85. The process is relatively fast, and it takes place in ~ 90 μ s and in the extracellular half-channel, an hydrophilic environment and rich in charges, where the proton travel less than 4 Å to the Asp85 residue,⁸ assisted by a water molecule.⁹ The decay of M_{412} ($M_{412} \rightarrow N_{550}$ process) is a much longer process, ~ 5 ms, where the Schiff base is reprotonated by Asp96 residue, lying in the cytoplasmic half-channel, ~ 12 Å away.¹⁰ The proton will have to travel a longer distance through a hydrophobic environment scarce in water.

The rise and decay of the intermediate M_{412} were measured via flash photolysis in the presence of Au NRs whose localized surface plasmon modes were continuously excited by an NIR cw laser with an output of 808 nm. The flash photolysis measurements were carried out using a frequency doubled Nd:YAG nanosecond laser at 532 nm and a 10 Hz repetition rate as the pump; the monitoring light was provided by a cw Xe–Hg lamp. The lamp output was focused to a 5 mm diameter spot at the sample. The transmitted beam went through a monochromator (Acton research 300i) fixed at 412 nm, where the absorption maximum of the M_{412} intermediate was located. Single wavelength kinetics of the intermediate was

obtained via monitoring the change in absorption at 412 nm using a photomultiplier tube (Hamamatsu R1477-06). The signal was averaged over 3000 laser pulses by a LeCroy 9350 500 MHz oscilloscope. The pulse energy of 1 mJ at 532 nm was used to initiate the photocycle in all samples. The NIR cw laser at 808 nm with a beam diameter of 5 mm is overlapped to the monitoring light in the sample.

Gold NRs with aspect ratio (AR) 3.9 were synthesized,¹¹ and the excess of CTAB was eliminated by centrifugation. The longitudinal plasmon band of these NRs was located at 795 nm, in resonance with the NIR cw source. A wavelength in the NIR was chosen so that the NRs could be continuously and independently excited without interfering with the bR photocycle. NRs were added to bR solution in deionized water at pH 7, and the rise and decay of M_{412} were monitored as the NR plasmonic field was turned on. BRs are adsorbed onto gold nanorods via nonspecific interactions. For simplicity and easy demonstration of gold nanoparticle surface plasmon field effects on the proton pump process of the bR, we chose to analyze all the kinetics of M_{412} decay with a monoexponential function, and the R^2 values higher than 0.99 are achieved because, at the neutral pH 7, the amplitude of the slower M_{412} decay is relatively small and, in our case, it is less than 10% of the total. It was observed that pure bR solution was not affected by the cw NIR laser for intensity as high as 0.6 W/cm². On the other hand, as shown in Figure 1, with the presence of gold NRs in the sample,

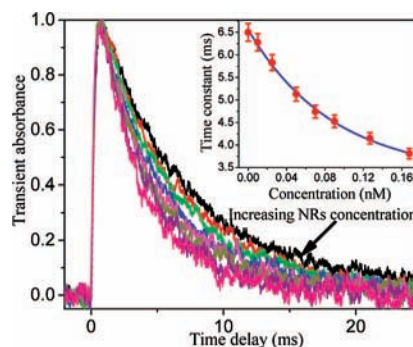


Figure 1. Dependence of the decay kinetics of M_{412} on Au NRs concentration in the presence of NIR laser irradiation with an intensity of 0.256 W/cm². Each decay lifetime value was obtained by fitting the decay kinetic with a single exponential function.

the decay time of M_{412} decreases as the concentration of NRs in the bR solution increases. The dependence of the time constant of decay on gold NR concentration is shown in the inset of Figure 1. However, under the same conditions, we did not observe an apparent effect on the formation of the M_{412} intermediate state.

In studying the dependence of M_{412} decay on gold NR concentration, the optical density ratio between the bR peak and the NRs

peak was always kept much lower than 1, even for the highest reported concentration. The intensity of the incident NIR cw laser for exciting the localized surface plasmon (LSP) modes was set at 0.256 W/cm^2 . We also demonstrated the dependence of M_{412} decay on the intensity of the NIR cw laser for the bR solution with 0.05 nM gold NRs. It is shown in Figure 2 that, with increasing intensity

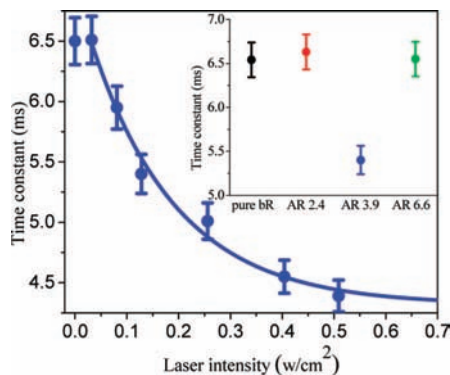


Figure 2. Dependence of the decay kinetics of M_{412} on cw NIR laser intensity in the presence of gold NRs (AR 3.9) with a concentration of 0.05 nM . Each value of time constant was obtained by fitting the decay kinetics with a single exponential function. The inset compares the time constants of pure bR and bR solutions mixed with gold NRs of different aspect ratios.

of NIR laser, the time constant of decay decreases. The inset compares the time constants from both pure bR and bR solutions mixed with gold NRs of different aspect ratios.

The inset of Figure 2 shows that the effect could only be observed when the LSP mode of the added Au NRs is in resonance with the wavelength of the used NIR laser. For Au NRs with aspect ratios of 2.4 and 6.6, their LSP modes are located at 650 and 1030 nm, respectively, which are dramatically detuned from the NIR laser wavelength and therefore their plasmon field could not be turned on and thus did not have any apparent effect on the lifetime.

Au NRs are known to efficiently convert the absorbed photons into heat through electron–electron and electron–phonon relaxations.¹² Therefore, the decrease in M_{412} lifetime observed in both studies (laser intensity and NRs concentration) in the presence of Au NRs could be due to temperature increase of bR, which accelerates the reprotonation processes. Here, the temperature increase of bR in our experiments could be estimated by utilizing the analytic equations derived by Cortie et al.¹³ If it is assumed that the heat transfer from Au NRs to the medium is fully conductive, the temperature increase of bR molecules in close proximity to Au NRs is only of the order of $10^{-2} \text{ }^\circ\text{C}$ under NIR laser irradiation with an intensity of 0.5 W/cm^2 , corresponding to the intensity value of the last data point shown in Figure 2. Furthermore, by using a thermocouple, we monitored the temperature change of bR solutions containing Au NRs of different concentrations under NIR laser irradiation with an intensity of 0.256 W/cm^2 , chosen to match the experimental situation shown in Figure

1. We did not observe the same dependence of temperature increase on gold concentration, which matches the observed decrease of the M_{412} lifetime presented in Figure 1. Consistent with our theoretical prediction, the maximum photothermal temperature increase resulting from the optical absorption of Au NRs is found to be $\sim 0.1 \text{ }^\circ\text{C}$. We believe that this small temperature increase will have a negligible effect on the reprotonation processes.

The above results can be summarized as follows: (A) only gold nanorods that can be excited by the laser used showed a decrease in the reprotonation decay kinetics. (B) This effect increases by increasing both the concentration of the effective NRs and the intensity of the cw laser inducing the plasmon field. (C) Thermal effects cannot explain the reduction in the M_{412} decay time.

The mechanism of the plasmonic field effect could be physical or chemical in nature. The physical effect could result from field coupling to the medium in which the transport of the proton from Asp96 to the Schiff base is taking place. This could explain the fact that the effect was observed in the reprotonation that occurs over a long distance of 12 \AA but not in the rapid intermolecular proton transfer process occurring between the protonated Schiff base and Asp85 in the deprotonation process. The latter process occurs also in a region which is probably more electrostatically shielded.

The other physical effect could be due to temperature. However, both theoretical prediction and experimental observation do not support this mechanism.

The chemical effect could result from photochemical changes. The fact that we do not observe a permanent change in the absorption spectrum after the experiment could suggest that it is not an irreversible photochemical effect. However, it could be a reversible photochemical change. Any photochemical effect would depend on the light intensity, which, in our experiment, could be large as a result of the plasmon field enhancements.

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