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Photoswitchable Catalysis by a Nanozyme Mediated by a Light-Sensitive Cofactor

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Supporting Information

ABSTRACT: The activity of a gold nanoparticle-based catalyst can be reversibly up- and down-regulated by light. Light is used to switch a small molecule between *cis*- and *trans*-isomers, which inhibits the catalytic activity of the nanoparticles to different extent. The system is functional in aqueous buffer, which paves the way for integrating the system in biological networks.

The visual phototransduction process is a beautiful example of the functional power of complex chemical networks.[1] The process is initiated by the photon-triggered *cis*−*trans* isomerization of the cofactor retinal, which activates a cascade of catalytic events eventually leading to an electrical output signal for processing by the nervous system. In the quest toward responsive synthetic chemical systems of increasing complexity, the availability of catalysts that can be regulated using an external trigger is an essential prerequisite.[2,3] The use of light is particularly attractive because it can be delivered very efficiently and with high temporal and spatial precision.[4] Although most attention has been focused on molecular catalysts equipped with light-sensitive molecular switches,[5−10] there is an increasing number of examples of catalytic systems of higher complexity that can be regulated with light.[11,12] In particular, nanoparticles functionalized with azobenzene-moieties have turned out to be very effective, which is mainly caused by the possibility to install innovative regulatory mechanisms (e.g., light-induced aggregation−dissociation) that are hard to achieve with molecular catalysts.[13−15] It has been shown recently that nanoparticle aggregation can also be controlled using an external light-sensitive molecule, which liberates a proton upon light-induced isomerization and in that way affects the aggregation state of the nanoparticles.[16,17] Here, we apply a different strategy to control the activity of catalytic nanoparticles relying on the use of a small light-sensitive cofactor which inhibits catalytic activity because it competes with the substrate for binding to the catalytic monolayer (Figure 1). This mechanism, together with the fact that the system functions in water, makes for a close analogy with the visual phototransduction process and opens the way to light-regulated hybrid networks composed of nanoparticles and enzymes.

Au NP **1** are gold nanoparticles (d = 1.6 ± 0.4 nm) passivated with a monolayer of C_9-thiols terminated with a 1,4,7-triazacyclononane (TACN)·Zn^{2+} headgroup (Figure 1).[18] Previously, we have shown that Au NP **1** and analogues catalyze very efficiently the transphosphorylation of 2-hydroxypropyl-4-nitrophenylphosphate (HPNPP), which is a model substrate for RNA-hydrolysis.[19,20] The system has been referred to as a nanozyme, because of its many analogies to enzymes: cooperativity between TACN·Zn^{2+}-complexes, Michaelis−Menten reaction kinetics, and down-regulation of catalysis by inhibitors that compete with the substrate for binding to Au NP **1**.[21−24] In particular, this latter aspect stimulated us to exploit the reversible interaction between a competitor and Au NP **1** as a tool to regulate catalytic activity in a similar way to what happens in the visual phototransduction process. This would require the use of a small light-sensitive molecule that would change the affinity for Au NP **1** upon photoisomerization. Our attention was drawn to commercially available 4-(phenylazo)-benzoic acid (**2**), because it combines a photoresponsive azobenzene and a carboxylic acid group, which is negatively charged at pH 7.0. UV−vis spectroscopy confirmed that also in the presence of Au NP **1** ([TACN·Zn^{2+}] = 20 μM, [HEPES] = 10 mM, pH = 7.0) **2** can be reversibly switched between two photostationary states (*trans*:*cis* = 35:65 after λ = 365 nm for 50 min, *trans*:*cis* = 77:23 after λ = 465 nm for 10 min) (Supporting Information). Throughout the paper, *cis*-**2** and *trans*-**2** refer to the photostationary states enriched in *cis*-**2** (65%) and *trans*-**2** (77%), respectively. Next, the relative affinities of both *cis*- and *trans*-**2** for Au NP **1** were determined by means of a competition experiment with the fluorescent probe 6,8-dihydroxy-1,3-pyrenedisulfonic acid (**3**) (Figure 2a). In these experiments, *cis*-**2** and *trans*-**2** were titrated separately to a buffered aqueous solution of Au NP **1** ([TACN·Zn^{2+}] = 20 μM) and **3** (8 μM). At this concentration, nearly all **3** was bound to Au NP **1** resulting in a nearly complete quenching of its fluorescence by the gold core.[25] The addition of increasing amounts of **2** resulted in the displacement of **3** from the monolayer surface, which could be detected by an increase in fluorescence intensity. The resulting displacement curves clearly showed that *trans*-**2** has a higher affinity (∼2.2 times) for Au NP **1** than to *cis*-**2**, which is a fundamental prerequisite for the use of **2** as a photoresponsive cofactor (Figure 2b).[26] The lower affinity of *cis*-**2** is ascribed to the increase in polarity of azobenzene upon *trans*−*cis* isomerization, which reduces favorable hydrophobic interactions with the apolar part of the monolayer.[13,27] Importantly, the complete reversibility of the interaction was demonstrated by measuring the fluorescence intensity after repetitive *trans*−*cis* and *cis*−*trans* isomerizations (Figure 2c). It is noted that the quantitative interpretation of these measurements required a correction for the photo-

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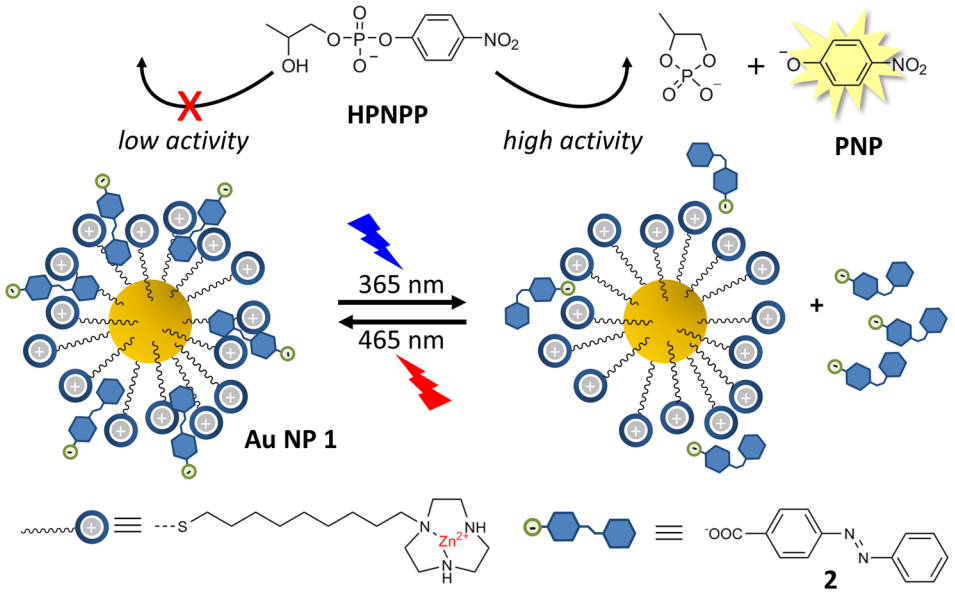

Figure 1. Light-induced *cis*–*trans* isomerization of **2** changes its affinity for Au NP **1**, which affects the transphosphorylation rate of HPNPP.

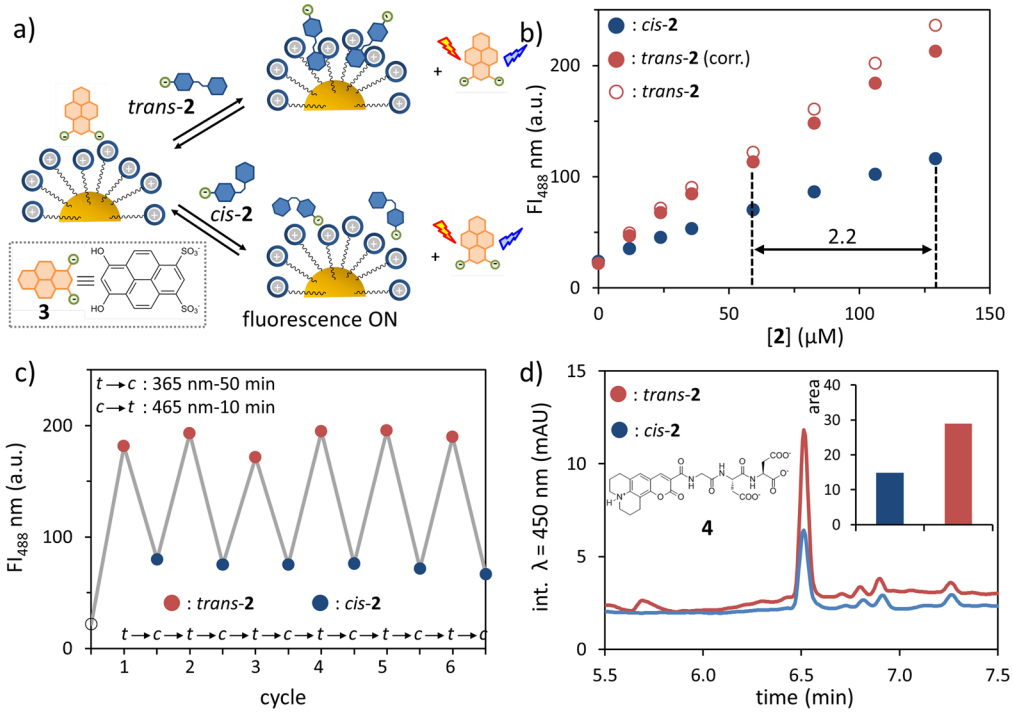

Figure 2. (a) Schematic representation of the competition experiments between fluorophore **3** and *cis*- and *trans*-**2** for binding to Au NP **1**. (b) Increase in fluorescence intensity as a function of the concentration of **2**. To permit comparison, the fluorescence intensity of *trans*-**2** was corrected for the intrinsic difference in fluorescence intensity of **3** in the presence of *trans*- and *cis*-**2**. (c) Fluorescence intensity after repetitive irradiations (365 nm for 50 min, 465 nm for 10 min) of a solution containing Au NP **1**, **2** and **3**. The intensity is corrected for bleaching of **3** (see Supporting Information). (d) Peak area of probe **4** (see Supporting Information) in the chromatograms of the dialysate after ultrafiltration of a solution containing Au NP **1**, probe **4**, and either *trans*- and *cis*-**2**. All experimental conditions are given in the Supporting Information.

bleaching of probe **3** upon irradiation at 465 nm (Supporting Information). Additional direct evidence that the *cis*- and *trans*-isomers of **2** displace a surface-bound molecule to a varying degree was obtained from ultrafiltration experiments followed by LC-MS measurements.[28] In these experiments, equal amounts of either *cis*- or *trans*-**2** (100 μM) were added to a solution of Au NP **1** saturated with probe **4** (4.8 μM, Figure 2d) after which ultrafiltration using a 10 kDa MW cutoff PES-membrane was used to separate free from surface-bound molecules. LC-MS measurements of the filtrate showed a 2 times higher concentration of **4** in the sample to which *trans*-**2** was added compared to the *cis*-**2** sample, which is in agreement with the data obtained from fluorescence measurements (inset of Figure 2d).

At this stage, we also verified through a series of analysis that Au NP **1** was not subject to structural alterations as a result of extensive irradiation. ^{1}H NMR and Cu^{2+}-titrations confirmed that 5 irradiation cycles did not affect the structural and

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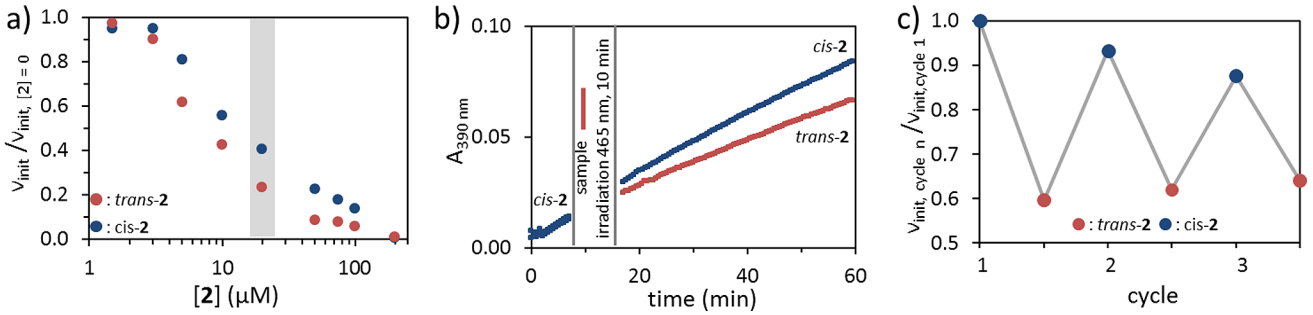

Figure 3. (a) Plot of the initial rate (normalized on the rate for [**2**] = 0 μM) of the transphosphorylation of HPNPP as a function of the concentrations of *cis*- and *trans*-**2**. (b) Increase in absorbance as a function of time for samples that were (red) and were not (blue) irradiated at 465 nm for 10 min after 7 min of reaction. (c) Plot of the initial rates (normalized on the initial rate of the first kinetics) for several irradiation cycles. Protocols and experimental conditions are given in the Supporting Information.

functional properties of the organic monolayer, whereas TEM, DLS, and UV−vis measurements confirmed the integrity of the inorganic core (Supporting Information).

We then proceeded with a study of the inhibitory effect of the *cis*- and *trans*-isomers of **2** on the catalytic activity of Au NP **1** in the transphosphorylation of HPNPP (Figure 1). The reaction can be conveniently followed by UV−vis spectroscopy by measuring the increase in absorbance at 390 nm (which corresponds to the isosbestic point of *cis*- and *trans*-**2**) originating from the liberated *p*-nitrophenolate anion. It is noted that the Au NP metal component itself is not involved in the reaction. During the course of numerous studies using Au NP **1** or analogues, the reduction of *p*-nitrophenol to *p*-nitroaniline, which is known to be catalyzed by Au metal nanoparticles,[29] has never been observed.[19,20] Inhibition studies were performed by measuring the initial reaction rate at different concentrations of either *cis*- or *trans*-**2**. Comparison of the inhibition curves showed an enhanced inhibitory capacity of *trans*-**2** compared to *cis*-**2**, which is in line with the results of the displacement experiments (Figure 3a).

In particular, between 5 and 20 μM of **2**, a significant difference was observed. From this range, a 20 μM concentration of **2** was chosen, because the lower reaction rates at higher inhibitor concentrations would increase the available time frame for detecting changes in activity upon irradiation. In an initial experiment, we followed the course of the reaction after adding HPNPP to a buffered solution containing Au NP **1** and *cis*-**2**. After 7 min, the cuvette was irradiated for 10 min at 465 nm after which the measurement was continued together with a sample that was not irradiated. Whereas prior to irradiation both rates were obviously the same, a lower activity was clearly observed for the irradiated sample. This indicated that the *cis*−*trans* isomerization of **2** resulted in a down-regulated activity of Au NP **1** (Figure 3b). Objectively, the observed difference in rate is not large, but it is in line with what can be expected based on the relative affinities of *cis*- and *trans*-**2** for Au NP **1**. It is worth reminding that **2** itself has a relatively low affinity for Au NP **1** and, also importantly, that the photostationary states are not very well resolved. The fact that the system is able to respond to the isomerization of **2** anyway illustrates the sensitivity of this approach to regulate the catalytic activity of Au NP **1** with light. The same experiment performed in the absence of the inhibitor **2** obviously resulted in higher rates, but, importantly, irradiation of the sample had in this case no effect on the reaction rate (Supporting Information). Yet, although promising, this procedure proved not very suitable for multiple up- and down-regulation cycles. This was mainly caused by the accumulation of the cleavage product *p*-nitrophenolate in the system, which strongly absorbs in the same region as azobenzene **2**. As a result, we observed a strong reduction in the switching efficiency of **2** as the reaction proceeded. For this reason, we performed the experiments in a different way by adding the substrate HPNPP each time after isomerization of **2**. Thus, a large stock solution of Au NP **1** (20 μM) and **2** (20 μM) in HEPES (10 mM, pH = 7.0) was prepared and subjected to irradiation cycles to induce isomerization. After each irradiation, a sample was transferred to a cuvette, HPNPP (100 μM) was added and the increase in absorbance at 390 nm was measured. This had also a second advantage that the initial rates could be quantitatively compared between different cycles. In total, 3 cycles were performed and the relative initial rates clearly demonstrated that the catalytic activity of the system can be reversibly up- and down-regulated using light (Figure 3c). Isomerization to the *trans*-isomer in all cases resulted in an (expected) decrease in activity, which was restored upon isomerization to the *cis*-isomer. In the absence of **2**, reversible up- and down-regulation was never observed (Supporting Information). The slight decrease in performance over multiple cycles is a side effect originating from a degradation of the HEPES-buffer upon irradiation (Supporting Information).[30,31] In that respect, we want to stress that this is one of the few examples of a light-modulated Au NP system that is functional in aqueous buffer[17] and thus marks a significant step toward the integration of such systems in biological networks.

In conclusion, we have shown that a small light-sensitive molecule can act as a cofactor to regulate reversibly the catalytic activity of a nanosystem. Similarly to what happens in the visual phototransduction process, light irradiation causes a structural change in the cofactor which affects the affinity for the catalytic site. Evidently, the efficiency of the presented system is still low compared to natural systems and also compared to previously reported nanoparticle-based catalysts that operate in organic solvents. However, the fact that the light-sensitive molecule interacts with the nanoparticle through noncovalent interactions in water represents a significant step forward. The use of small, easily accessible molecule significantly facilitates optimization compared to related systems in the literature in which the light-sensitive unit is covalently attached to the monolayer. In addition, we have shown that light can be used to displace surface bound molecules, which is of interest for the development of innovative delivery systems and materials.

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■ ASSOCIATED CONTENT

Supporting Information

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Materials and instrumentation, experimental protocols, control experiments as mentioned in the paper (PDF)

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Notes

The authors declare no competing financial interest.

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