

SUPPORTING INFORMATION

Photoswitchable catalysis by a nanozyme mediated by a light-sensitive cofactor

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1. Materials and instrumentation

The synthesis and characterization of Au NP **1** has been described elsewhere, but has anyway been reported here again (SI-2) to verify the stability of the system against irradiation.¹ The stock solution of Au NP **1** (Zn²⁺-free) was stored at 4°C in mQ water. The TACN head group concentration of Au NP **1** was determined from kinetic titrations using Zn(NO₃)₂ as reported previously.² Zn(NO₃)₂ was obtained as an analytical grade product. The buffer 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 4-(phenylazo)benzoic acid (**2**) and 6,8-dihydroxy-1,3-pyrenedisulfonic acid (**3**) were purchased from Sigma Aldrich and used without further purification. The synthesis and characterization of probe **4** has been described previously.³

The concentrations of **2** and **3** were determined by weight. The concentration of **4** was determined both by weight and UV-Vis spectroscopy using $\epsilon_{450} = 45000 \text{ M}^{-1} \text{ cm}^{-1}$ at pH 7 as the molar extinction coefficient. Stock solutions were always freshly prepared in DMSO/H₂O = 5/2 (**2**), mQ water (**3**) or EtOH (**4**), respectively.

UV-Vis spectra were recorded on a Varian Cary50 spectrophotometer equipped with thermostatted multiple cell holders. Fluorescence measurements were recorded on a Varian Cary Eclipse fluorescence spectrophotometer equipped with a thermostatted cell holder. The quantification of the two isomers was performed with an Agilent Technologies 1290 Infinity LC equipped with a quadrupole MS. The UV lamp used for the irradiation was a spectroline, MODEL ENF-240C/FE 230V, 50Hz, 0.17 AMPS. The irradiation at 465 nm was performed with LED lights, RoHS Compliance Guirlande Electrique 80 Lampes 230V, 50Hz, 20 mA.

2. Stability of Au NP 1 upon irradiation

After 5 irradiation cycles (1 cycle = 365 nm for 1h and 465 nm for 10 min) of a solution of Au NP 1 (1 mL, [TACN] = 1 mM) in mQ water, the sample was analysed by UV-Vis and ^1H -NMR spectroscopy, DLS, and TEM, and compared to a non-irradiated control sample.

No difference in the UV-Vis spectra was detected suggesting that Au NP 1 does not aggregate (Figure S1a). This was also confirmed by DLS, which showed no changes in the hydrodynamic diameter before and after irradiation (Figure S1b). Finally, TEM analysis showed no significant changes in the diameter of the gold core (Figure S1c).

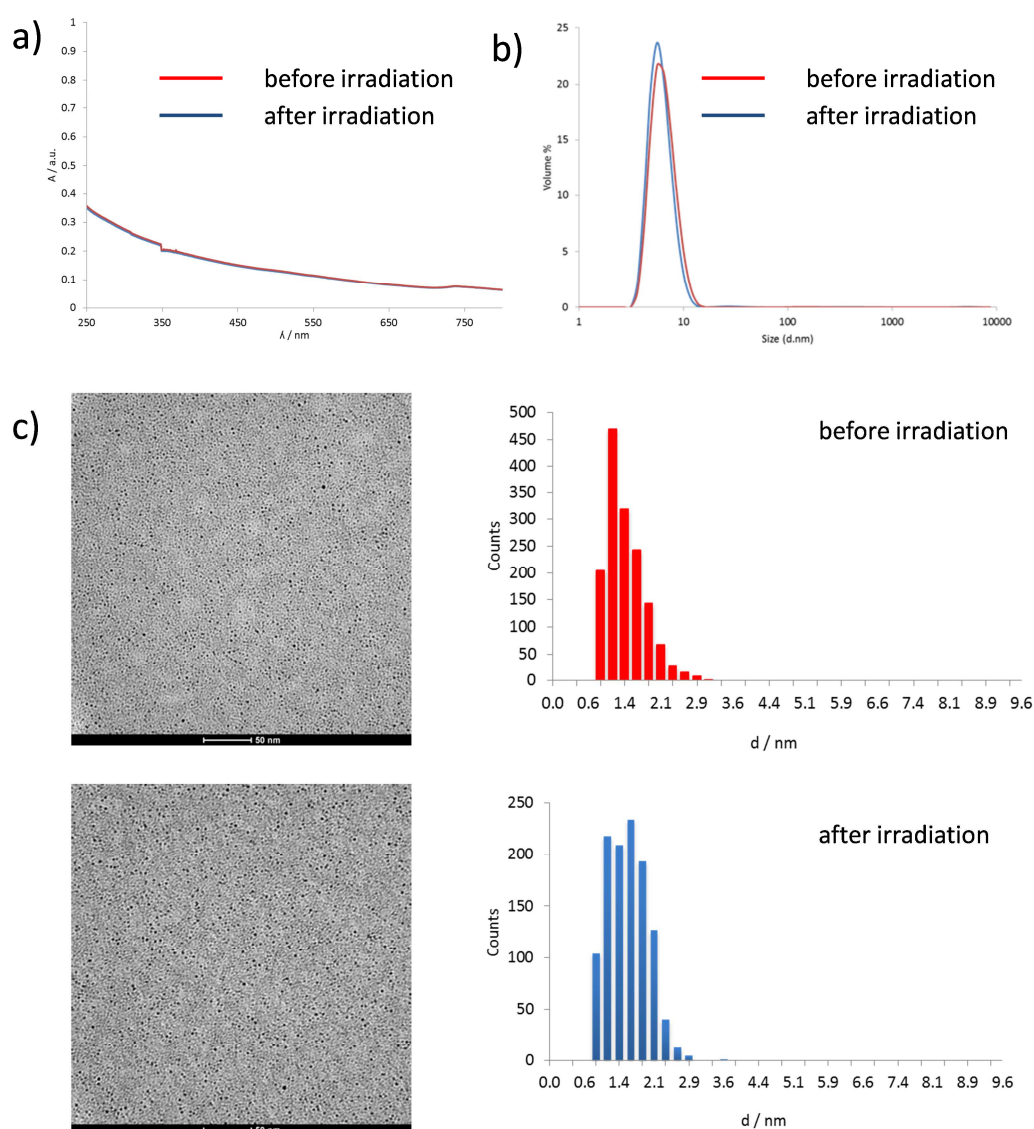


Figure S1. a) UV spectra of a solution of Au NP 1 (20 μM) before (red) and after (blue) irradiation (1 cycle = 365 nm 1h 465 nm 10 min). b) DLS of Au NP 1 before (red) and after (blue) irradiation (1 cycle = 365 nm 1h 465 nm 10 min) and c) TEM images before (red) and after (blue) irradiation (1 cycle = 365 nm 1h 465 nm 10 min).

The stability of the organic monolayer was verified by ^1H -NMR spectroscopy and Cu^{2+} titrations of the TACN head groups followed by UV-Vis spectroscopy. No detectable differences were observed in the ^1H -NMR spectra before and after irradiation (Figure S2a). Cu^{2+} -titrations were performed to determine the integrity of the head groups relying on the possibility to detect formation of the $\text{TACN}\cdot\text{Cu}^{2+}$ complex by UV-Vis spectroscopy.⁴ A solution of $\text{Cu}(\text{NO}_3)_2$ was titrated to 1 mL of a solution containing Au NP **1** ($[\text{TACN}] = 20\ \mu\text{M}$) –before and after irradiation– in HEPES (10 mM, pH 7.0). The UV-Vis spectrum was recorded after each addition and the absorbance at 264 nm was monitored as a function of the number of equivalents of Cu^{2+} . No difference was observed between the two samples, suggesting that irradiation does not affect the ability of TACN to complex metal ions (Figure S2b).

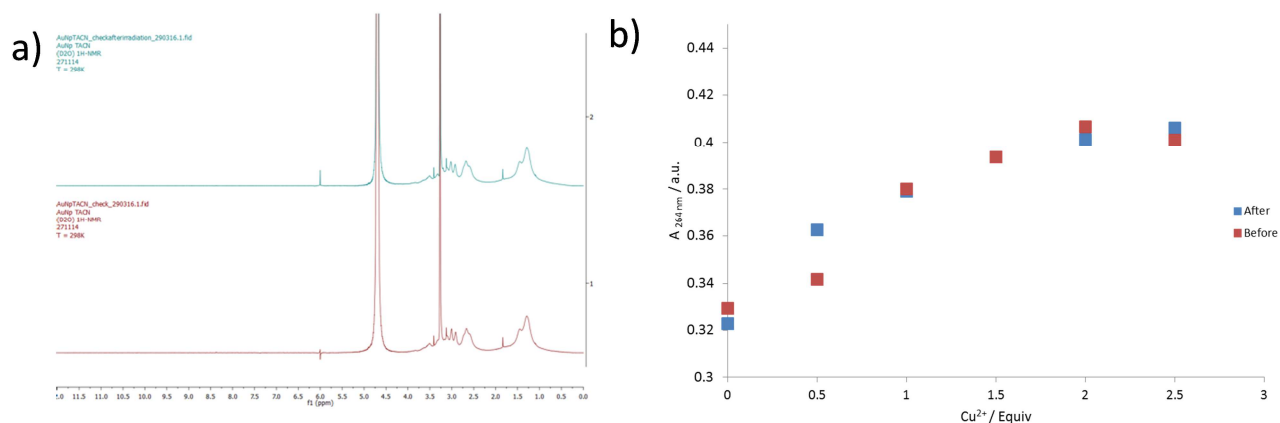


Figure S2. a) ^1H -NMR spectra of Au NP **1** in D_2O , (D_2O , 500 MHz, 298K) and b) Cu^{2+} -titrations of the TACN-head groups measured by UV-Vis before (red) and after (blue) irradiation. Conditions: $[\text{HEPES}] = 10\ \text{mM}$, $\text{pH} = 7.0$, $T = 25^\circ\text{C}$.

3. Light-induced isomerization of 4-(phenylazo)benzoic acid (**2**)

A. Isomerization in the absence of Au NP 1

HPLC revealed that **2** (as received) had a *trans*:*cis* ratio of 98:2 (Figure S3a). The peak intensities in the HPLC chromatogram were measured at 390 nm, which is the isosbestic point in the UV-Vis absorption spectrum (Figure S3b). The peak of the *trans*-isomer (r.t. = 2.3 min) was then used to determine the change in concentration upon irradiation (Figure S4a). It is important to note that the peak intensity was measured at $\lambda = 326$ nm, which corresponds to the absorption maximum of *trans*-**2**, rather than $\lambda = 390$ nm to increase precision. This is why the peak of *trans*-**2** is always higher than the peak of *cis*-**2**. *Trans*-*cis* isomerization was induced by irradiating a solution of **2** (100 μ M) in aqueous buffer ([HEPES] = 10 mM, pH = 7.0, T = 25 °C) at 365 nm. After 50 minutes a photostationary state was reached with a *trans*:*cis* ratio corresponding to 34:66 (Figure S4a+b). The photochemical back-isomerization required irradiation at 465 nm for 10 min and lead to a photostationary state with a *trans*:*cis* ratio of 75:25 (Figure S4a+b). The thermal *cis*-*trans* isomerization (dark, T = 25 °C) gave the initial *trans*:*cis* ratio of 98:2 after 2 days.

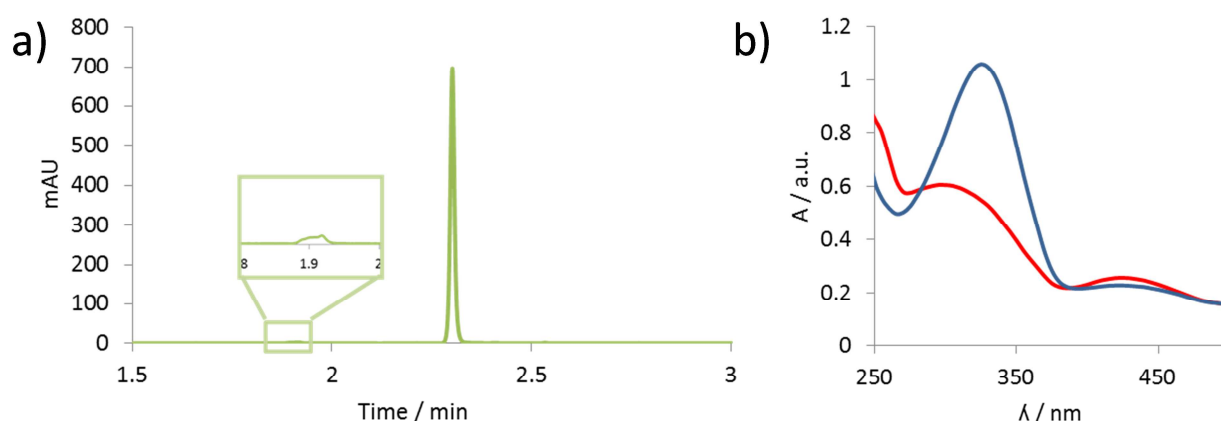


Figure S3. a) HPLC chromatogram ($\lambda = 390$ nm) indicating *cis*-**2** (r.t. = 1.9 min) and *trans*-**2** (r.t. = 2.3 min). Conditions: column Zorbax Eclipse Plus SB-C8, gradient: 5-95 %B in 3 min, 0.8 mL/min; A = H₂O+0.1% HCOOH, B = ACN+0.1% HCOOH, Sample: [**2**]=100 μ M, [HEPES] = 10 mM, pH = 7.0, T=25°C. b) Characteristic UV-Vis spectra of *cis*-**2** (red) and *trans*-**2** (blue) showing an isosbestic point at 390 nm.

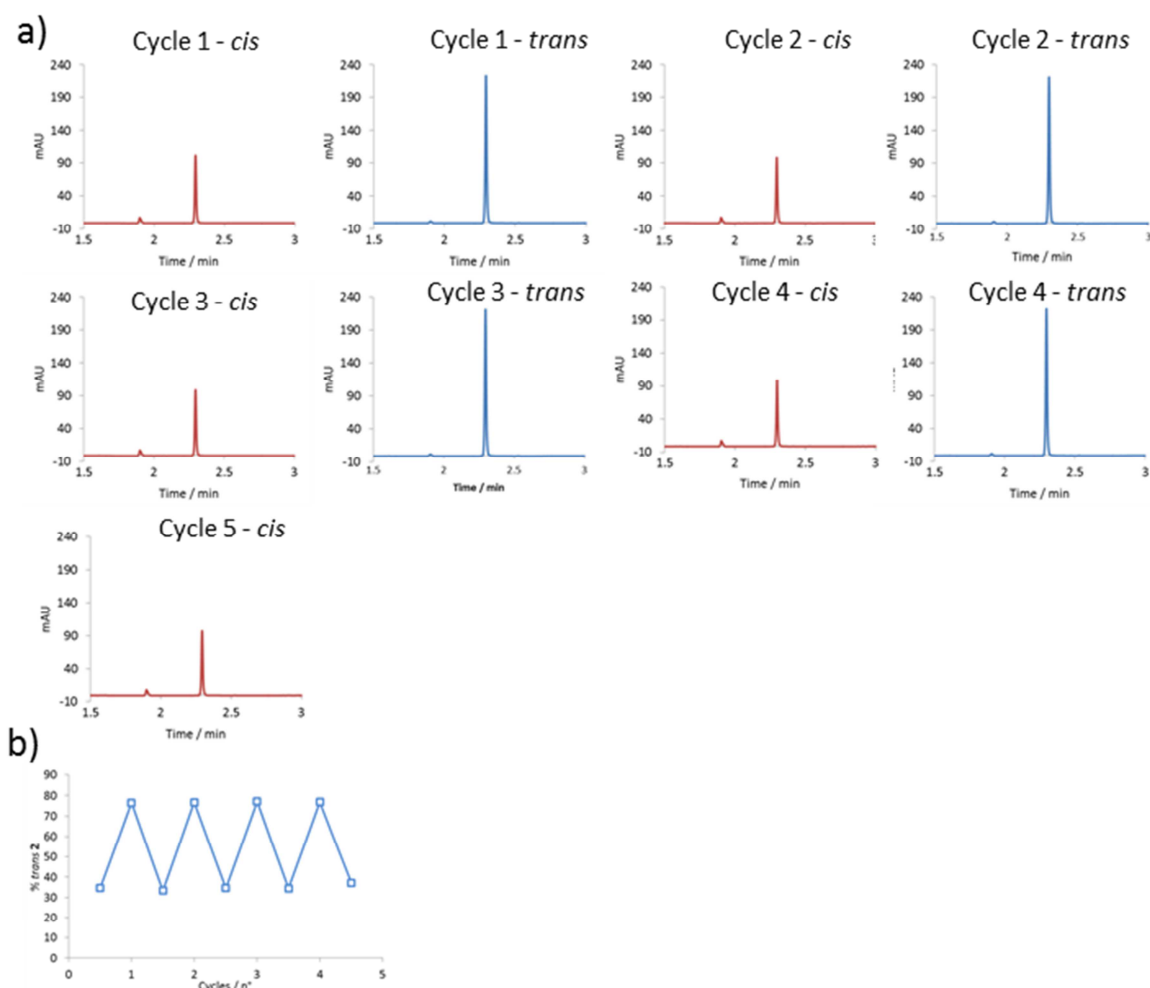


Figure S4. a) HPLC chromatograms used to determine the concentration of *trans*-**2** after several cycles of alternating irradiations at 365 nm for 50 min and 465 nm for 10 min, respectively. Conditions: column Zorbax Eclipse Plus SB-C8, gradient: 5-95 %B in 3 min, 0.8 mL/min; A = H₂O+0.1% HCOOH, B = ACN+0.1% HCOOH measured at 326 nm. Sample: [**2**]=20 μ M, [HEPES] = 10 mM, pH = 7.0, T=25°C. b) Relative ratio of *trans*-**2** after each irradiation.

B. Isomerization in the presence of Au NP 1

The same procedure was repeated in the presence of Au NP **1** ([TACN·Zn²⁺] = 20 μ M). No significant effect on the isomerization of **2** were observed, both in terms of rate and composition. *Trans*:*cis* ratios of 35:65 and 77:23 were determined for the two photostationary states after irradiation at λ = 365 nm for 50 minutes and λ = 465 nm for 10 minutes, respectively (Figure S5a). Also in this case a complete reversibility was observed (Figure S5b).

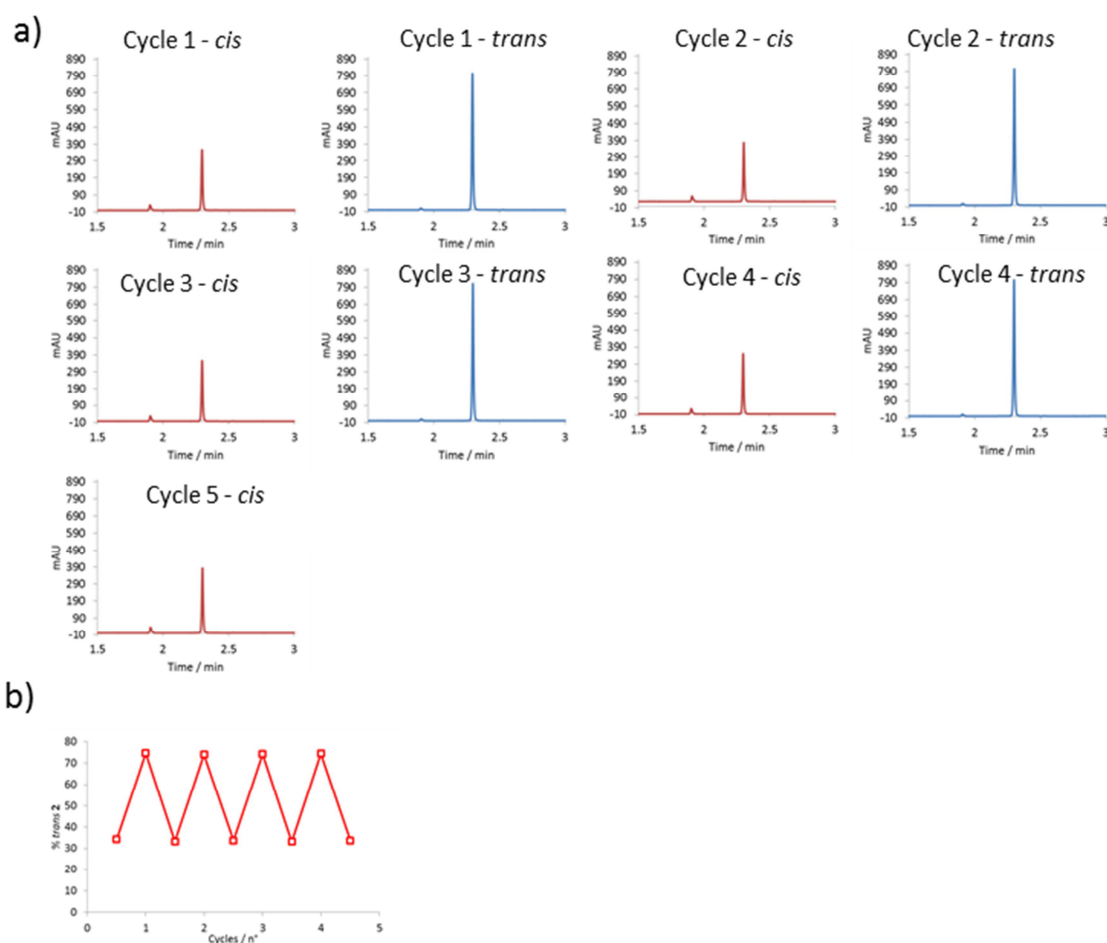


Figure S5. a) HPLC chromatograms used to determine the concentration of *trans*-2 after several cycles of alternating irradiations at 365 nm for 50 min and 465 nm for 10 min, respectively, in the presence of Au NP 1 (20 μ M). Conditions: column Zorbax Eclipse Plus SB-C8, gradient: 5-95 %B in 3 min, 0.8 mL/min; A = H₂O+0.1% HCOOH, B = ACN+0.1% HCOOH measured at 326 nm. Sample: [Au NP 1] = 20 μ M, [2]=100 μ M, [HEPES] = 10 mM, pH = 7.0, T=25°C. b) Relative ratio of *trans*-2 after each irradiation.

4. Competition experiments to determine the relative affinities of *trans*- and *cis*-**2** for Au NP **1**

A. Fluorescence measurements

The displacement experiments were performed by measuring the fluorescent intensities after adding consecutive amounts of stock solutions of either *cis*- or *trans*-**2** to a 3 mL solution of Au NP **1** (20 μ M) and **3** (8 μ M) in aqueous buffer ([HEPES] = 10 mM, pH = 7.0). Instrument settings: λ_{ex} = 407 nm, λ_{em} = 488 nm, slit: 5/5 nm. The fluorescent intensities were measured after stabilization of the signal (about 10 minutes) (Figure S6). See Figure 2b of the manuscript.

Considering that *cis*- and *trans*-**2** have different absorption spectra, we wanted to exclude that this would be the source of the observed differences in the displacement curves. The intrinsic effect of the presence of *cis*- and *trans*-**2** on the fluorescence intensity of **3** was determined by titrating a solution of **3** (8 μ M) in aqueous buffer ([HEPES] = 10 mM, pH = 7.0) with either *cis*- or *trans*-**2** and measuring the fluorescence intensity after each addition. The difference between the two curves was then used to correct the displacement curve of *trans*-**2**. This effect was only marginal, indicating that the different displacement curves of *cis*- and *trans*-**2** indeed originate from their different affinity for Au NP **1**. See Figure 2b of the manuscript.

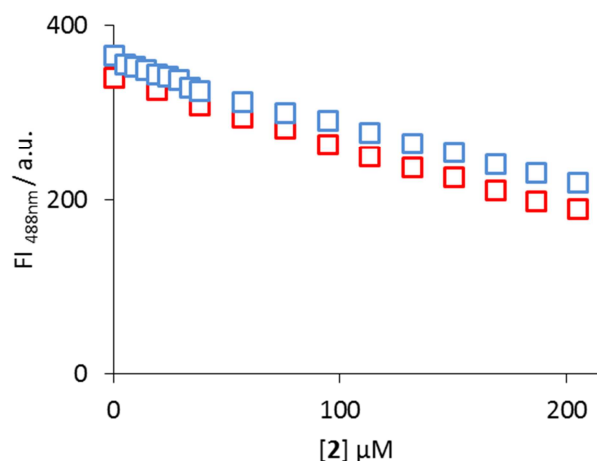


Figure S6. intrinsic difference in fluorescence intensity of **3** in the presence of *trans*- (blue) and *cis*-**2** (red)

B. Ultrafiltration experiments

Previously, we have shown that ultrafiltration can be used to separate molecules bound to the surface of Au NP **1** from molecules that are free in solution.⁵ Separation relies on the use of membranes with a defined molecular weight (MW) cut-off. In this case Vivaspin PES membranes with a MW-cut-off of 10 kDa were used.

A 2.1 mL solution of Au NP **1** ([TACN]=20 μ M) and **4** (4.8 μ M) in aqueous buffer ([HEPES] = 10 mM, pH = 7, T = 25 °C) was divided in aliquots of 500 μ L. To the first aliquot, *cis*-**2** (100 μ M) was added and the solution was shaken for 5 min. Then the solution was filtered at 12 rpm for 15 s leading to a reduction in volume corresponding to 20%. The filtrate was analysed by LC/MS. After that the solution of *cis*-**2** was irradiated for 10 min at 465 nm to obtain the *trans*-**2**. *Trans*-**2** (100 μ M) was added to a second aliquot of the Au NP solution and the solution was shaken for 5 min and filtered in the same way as described above. The filtrate was analysed by LC/MS. The concentration of **4** in the filtrate was detected both by absorbance (λ = 450 nm) and SIM mode (selected-ion monitoring mode) to unambiguously assign the peak. See Figure 2d in the manuscript

5. Reversible displacement of probe 3 from Au NP 1 regulated by light

Trans-**2** (100 μ M) was added to a solution of Au NP **1** (20 μ M) and **3** (8.1 μ M) in aqueous buffer ([HEPES] = 10 mM, pH = 7.0, T = 25 $^{\circ}$ C) and the fluorescence intensity at 488 nm (λ_{ex} = 407 nm) was measured. The the solution was then irradiated at 365 nm for 50 minutes to induce *trans*-*cis* isomerization after which the fluorescence intensity was measured again. After that, the solution was irradiated at 465 nm for 10 min to return to *trans*-**2**. This cycle was repeated 4 more times. The results are provided in Figure S7.

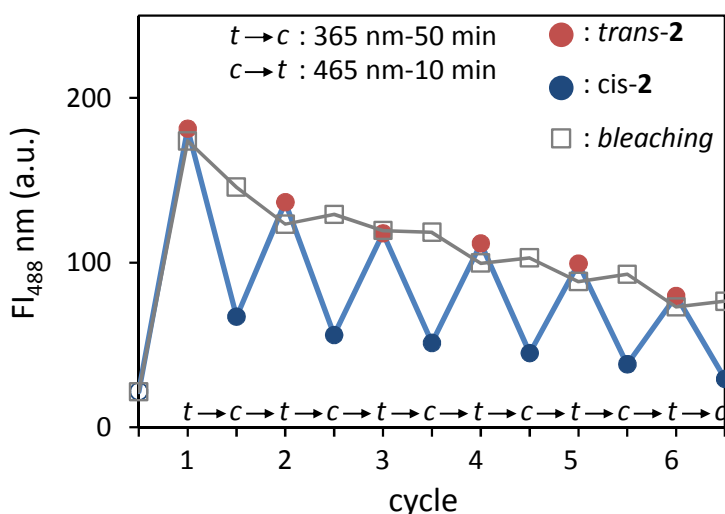


Figure S7. Fluorescence intensity after repetitive irradiations (365 nm for 50 minutes, 465 nm for 10 minutes) of a solution of Au NP **1** (20 μ M), **2** (100 μ M) and **3** (8.1 μ M). The grey line indicates the bleaching of **3**.

From the results it is evident that after each cycle the fluorescence intensity for each state diminishes. This is a consequence of photobleaching of probe **3**, which was demonstrated by a control experiment in which the same irradiation cycles were carried out in the presence of 4-phenylazophenol (100 μ M). This compound is very similar to **2**, but has no affinity for Au NP **1** because of the absence of the carboxylate group. Thus, while the presence of this compound ensures the presence of the same chromophore, its *cis*-*trans* isomerization does not affect the amount of **3** in solution. In order to promote the displacement of the same amount of **3** as obtained for **2**, guanosine 5'-triphosphate (GTP, 2.6 μ M) was added as an independent competitor. The same irradiation cycles were performed as reported above, and the fluorescence intensity was measured after each irradiation. The resulting profile (grey line in Figure 2c) reflects the photobleaching of **3** and was used to correct the intensities reported in Figure S6 (see Figure 2c in the manuscript).

6. Catalytic experiments

A. Inhibition experiments

The catalytic activity of Au NP **1** in the presence of increasing amounts of either *cis*- or *trans*-**2** was carried out by measuring the increase in absorbance at 390 nm upon the addition of HPNPP (100 μ M) to a solution containing Au NP **1** ([TACN] = 20 μ M) and different concentrations of either *cis*- or *trans*- **2** (ranging from 1.5 to 200 μ M) ([HEPES] = 10 mM, pH = 7.0, T = 25 $^{\circ}$ C). Kinetics were measured for 30 minutes (linear regime) and the rate (dA/dt) was determined by fitting the data to a straight line. Rates were normalized on the rate measured in the absence of **2** leading to the inhibition curves provided in Figure 3a of the manuscript.

B. In-situ down-regulation of catalytic activity

HPNPP (100 μ M) was added to a 900 ml solution of Au NP **1** ([TACN] = 20 μ M) and *cis*-**2** (20 μ M) in aqueous buffer ([HEPES] = 10 mM, pH 7.0). The increase in absorbance at 390 nm was recorded for 7 minutes after which the solution was irradiated for 10 min at 465 nm. Measurement of the absorbance at 390 was then continued for an additional 40 minutes. The blank consisted of the same experiment without the irradiation step (See Figure 3b of the manuscript). Both experiments were also repeated in the absence of **2** (Figure S8).

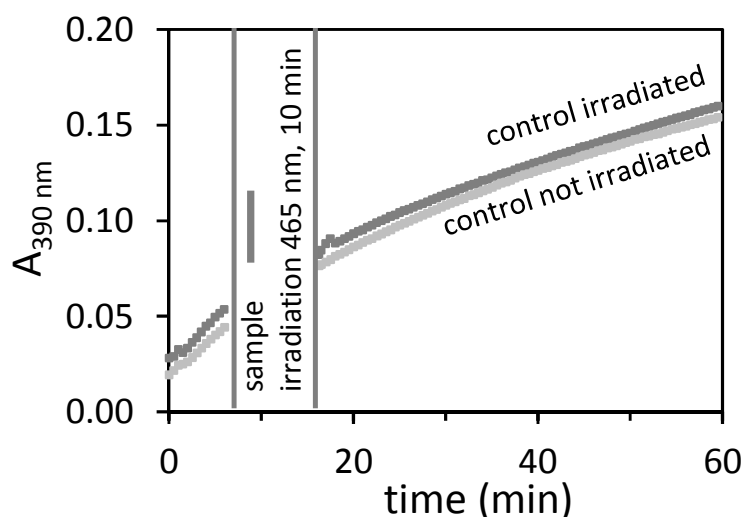


Figure S8. Increase in absorbance as a function of time for control sample that were (dark grey) and were not (light grey) irradiated at 465 nm for 10 minutes after 10 minutes of reaction. These experiments are performed in the absence of **2**.

C. Reversible up- and down-regulation of catalytic activity

A large stock solution of 10.5 mL was prepared containing Au NP **1** ([TACN] = 20 μ M), **2** (20 μ M) and HEPES (10 mM, pH = 7.0) and irradiated for 50 min at 365 nm to obtain *cis*-**2**. Then, 900 μ L of this solution was transferred to a UV cuvette and 100 μ L of a 1 mM HPNPP stock solution was added (final HPNPP conc. = 100 μ M). The transphosphorylation of HPNPP was followed by measuring the increase in absorbance at 390 nm for 30 minutes. The initial stock solution was irradiated at 465 nm for 10 min to induce *cis-trans* isomerization, after which 900 μ L was transferred to a cuvette to which 100 μ L of a 1 mM HPNPP stock solution was added (final HPNPP conc. = 100 μ M) and the catalytic activity measured as before. These cycles were repeated 2 more times (Figure S9). Rates were normalized on the value obtained for the first sample.

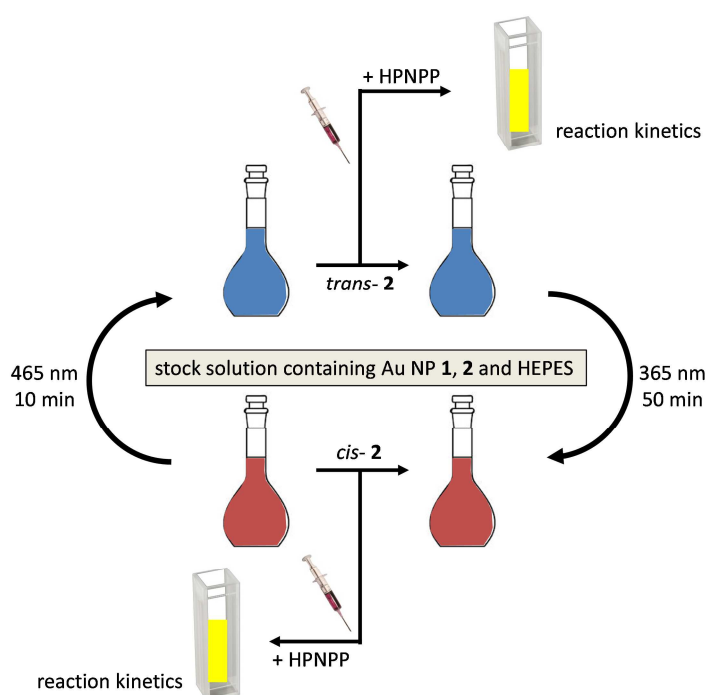


Figure S9. Schematic representation of the experiment.

To study the intrinsic effect of (prolonged) irradiation on the activity of the system (*i.e.* effects other than those originating from *cis-trans* isomerization), the experiment was repeated in the absence of **2** and in the absence of **2** and HEPES in the stock solution (which was added at the same time as HPNPP) (Figure S10). Also in these cases the rates were normalized on the value obtained for the first sample. It is noted that the absolute rates are obviously higher in these experiments because the inhibitor **2** is not present. An analysis of the results demonstrates that in the absence of **2** upregulation of catalytic activity never occurs. Furthermore, HEPES plays an important role in the general deactivation of the system, which hardly takes place when HEPES is absent during irradiation. Indeed, it is known that HEPES is light-sensitive.⁶

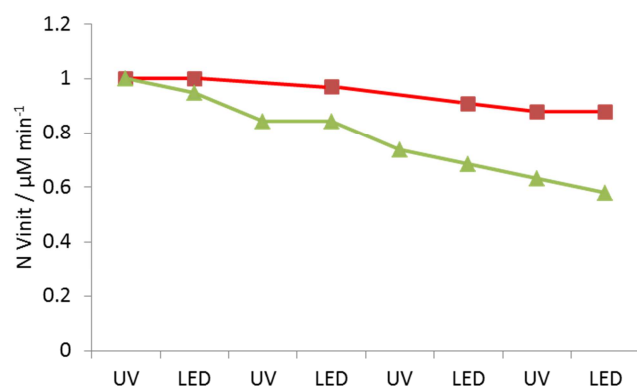


Figure S10. Normalized initial rate of HPNPP transphosphorylation promoted by Au NP **1** with (green) and without (red) the presence of HEPES in the stock solution. Conditions green: [Au NP **1**] = 20 μM , [HPNPP] = 100 μM , [HEPES] = 10 mM, pH = 7, Conditions red : [Au NP **1**] = 20 μM , [HPNPP] = 100 μM .

7. References

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