Development of Multiplexed Analysis for the Photocatalytic Activities of Nanoparticles in Aqueous Suspension

No Ah Lee[a,c], Soo Jin Kim[a], Bong-Jae Park[a], Hyun Min Park[b], Minjoong Yoon[*c], Bong Hyun Chung[d] and Nam Woong Song[*a]

[a]Center for Nanobio Convergence, Korea Research Institute of Standards and Science, 209 Gajeong-Ro, Yuseong-Gu, Daejeon 305-340, Rep. of Korea

[b]Center for Nano-Characterization, Korea Research Institute of Standards and Science, Daejon 305-340, South Korea

[c]Molecular/Nano Photochemistry & Photonics Lab., Department of Chemistry, Chungnam National University, 99 Daehak-Ro, Yuseong-Gu, Daejeon 305-764, Korea

[d]BioNanotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology and Nanobiotechnology Major School of Engineering, University of Science and Technology 111 Gwahang-Ro, Yuseong-Gu, Daejeon 305-806, Korea

[*]Corresponding authors:

Nam Woong Song, Ph.D.

e-mail: nwsong@kriss.re.kr, Tel: +82-42-868-5214, Fax:+82-42-868-5032

Minjoong Yoon, Ph.D.

e-mail: mjyoon@cnu.ac.kr, Tel: +82-42-821-6546, Fax:+82-42-821-8896

Supporting Information

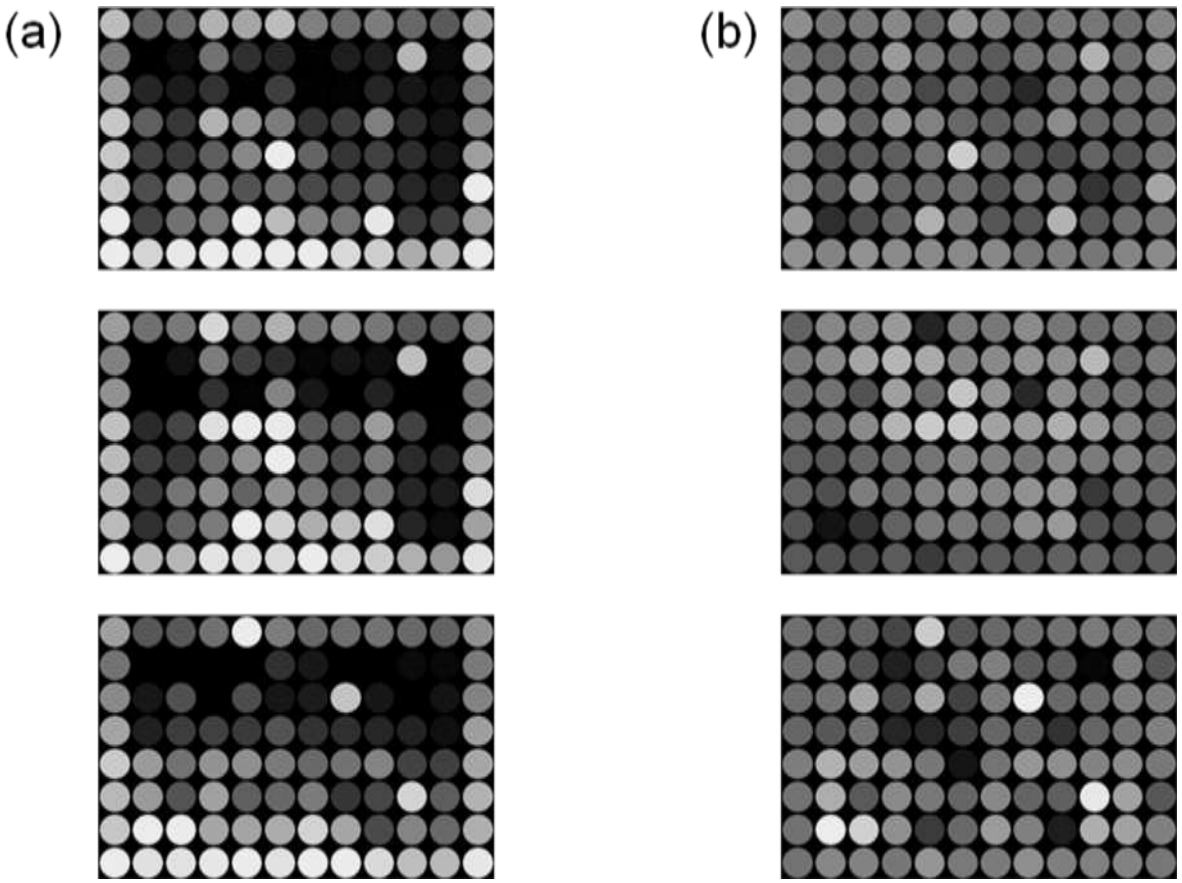

Fig. S1. (a) k_{app} values of NADH in each well depicted as a contrast image observed from three independent plates. (A brighter circle corresponds to the well with higher rate constant) (b) The reconstructed contrast image of the k_{app}'s corresponding to the images in the left column. – *Images in the first raw are the same ones as in Fig. 2 in the main text.*

S1. Experimental Parts

S1.1 Materials

TiO_2 as the photocatalytic nanomaterial was purchased from Evonic Co. LTD. (Aeroxide Degussa P25®) and was used without further purification. To obtain a stable suspension of TiO_2 NPs, 40 mg of TiO_2 powder was mixed into 10 mL of an aqueous NaOH (S8045, >98%, Sigma-Aldrich) solution of pH 10 stored in a 20-mL glass vial (ϕ = 26 mm). The solution was stirred using a 22-mm magnetic bar at 900 rpm for 24 h and the suspension was maintained for an additional 24 h without stirring to sediment large particles. Then 5 mL of the supernatant was sampled for further analysis. The concentration of the TiO_2 NP suspension

was determined as 2.1 g/L by measuring the weight of the 1 mL of the suspension after dehydration and comparing the UV absorbance at 310 nm with that of highly concentrated suspensions. The hydrodynamic diameter of TiO_2 NPs in suspension was measured as 160 ~ 190 nm using a particle size analyzer (ELS-Z, Otsuka electronics Co. Ltd.)

The stock solution of NADH for the PCA probe was prepared by dissolving 33 mg of NADH (Cat. No. 43423 Fluka. Co. LTD.) into 10 mL of deionized water (DIW> 18 $M\Omega \cdot cm^{-1}$) obtained from the Milli-Q reference water system production unit (Millipore Co.). The solution was then diluted by a factor of 5 ~ 80 to adjust the concentration adequately for the experimental condition.

Acetic acid/sodium acetate, sodium phosphate monobasic/sodium phosphate dibasic and sodium carbonate/sodium bicarbonate solutions of equal concentration were used for the preparation of pH buffers of pH 5 ~ 6, pH 6 ~ 8 and pH 9 ~ 11, respectively. All of the reagents for the preparation of pH buffers were purchased from Sigma-Aldrich as ACS reagent grade.

S1.2 Spectral Measurement procedure

Absorbance spectra of the TiO_2 suspension and NADH solution were recorded in the wavelength range between 200 and 500 nm using a small-volume (100 μL) absorbance cuvette (Hellma 105.201-QS) and a spectrophotometer (UV-1700, Shimadzu). The fluorescence spectrum measurement of the NADH solution was carried out using a scanning-type spectrofluorometer (Shimadzu RF-5301PC) with a small volume (100 μL) sample cuvette (Hellma 105.250-QS).

S2. Dependence of NADH fluorescence intensity on NADH and TiO_2 concentrations

To measure the oxidation rate of NADH by observing the fluorescence intensity, the

experiment should be performed in a concentration range exhibiting linear dependence of the fluorescence intensity. The concentration dependence of the fluorescence intensity differs according to the optical measurement geometry.[1] In this study, the top-read mode of fluorescence microplate reader was used, which entail both excitation and detection of the fluorescence through optical fibers located above a 96-well plate. In this optical geometry, the fluorescence intensity of NADH showed a linear dependence on NADH concentration up to 250 μM with a coefficient of determination close to 1 (R^2 = 0.995) (Table S1 and Fig. S2(a)). The fluorescence intensity exhibited saturation behaviour when the concentration was increased over 125 μM. Similar dependence of the NADH fluorescence intensity on the concentration could be observed in the presence of TiO_2 NPs (Table S1 and Fig. S2(b)). Such behaviour is due to the absorption of excitation light in the direction of propagation. Because the conjugate focal point of the detection fiber input lies at a certain depth within the NADH solution, the excitation light reaching that point is more attenuated by the absorption of NADH as the concentration increases. Based on these results, the maximum concentration of NADH was set to 125 μM.

Table S1 Fluorescence intensities of NADH observed at various concentrations of NADH and TiO_2 NPs

[NADH] (μM)	[TiO_2] (mg/L)					
	0	16	31.3	62.5	125	250
7.82	212.0	186.8	169.3	139.8	106.8	78.75
15.6	326.6	294.3	272.3	239.1	193.8	148.4
31.3	556.3	509.8	479.7	428.9	364.1	285.1
62.5	931.4	869.8	837.3	758.8	654.6	535.1
125	1,510	1,428	1,391	1,284	1,130	958.5
250	2,276	2,186	2,145	2,026	1,785	1,558
500	2,919	2,877	2,848	2,741	2,572	2,353
1,000	3,366	3,337	3,342	3,305	3,228	3,071

* pH value was adjusted to 8 with 5 mM phosphate buffer

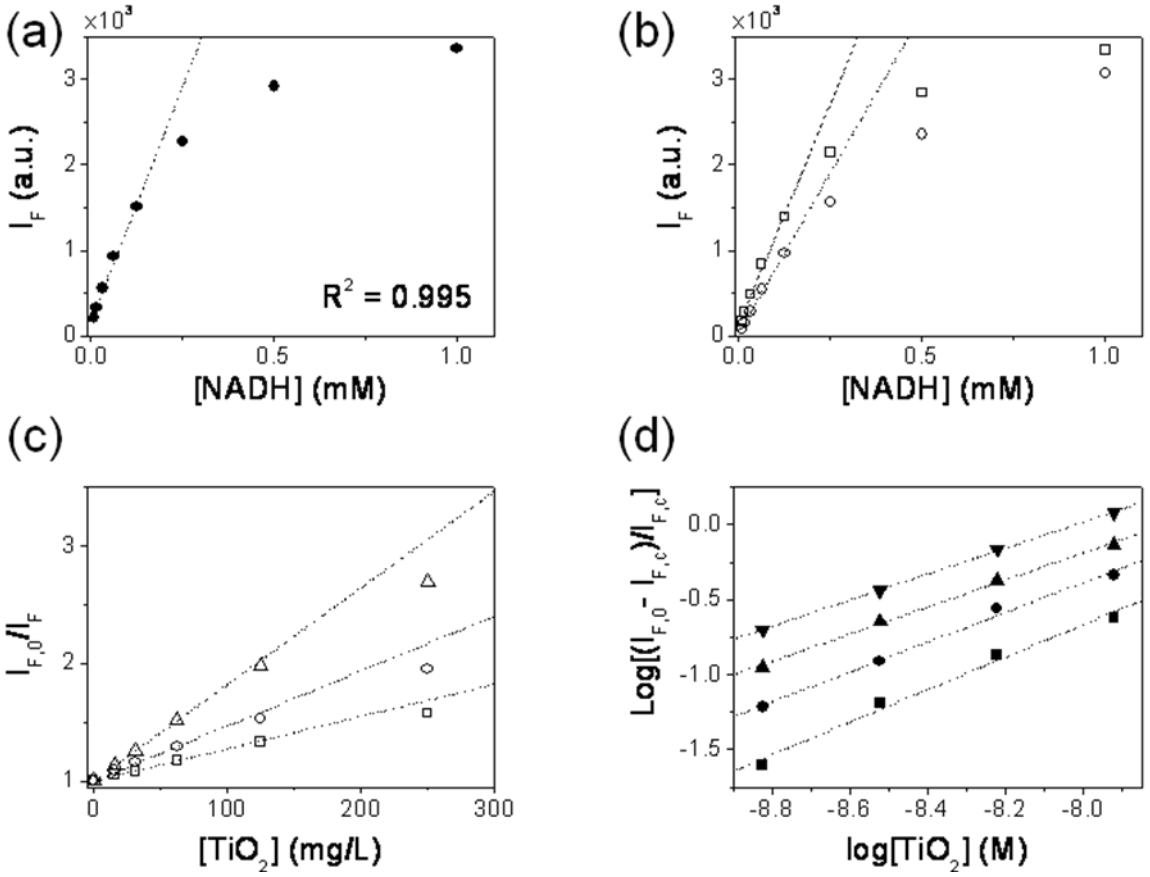

Fig. S2. (a) The dependence of fluorescence intensity on NADH concentration in the absence of TiO_2 NPs. (b) The dependence of the NADH fluorescence intensity on NADH concentration in the presence of TiO_2 NPs of (□) 31.3 and (○) 250 mg/L. (c) The dependence of NADH fluorescence intensity on the concentration of TiO_2 NPs with NADH concentration of (□) 125, (○) 31.3 and (△) 7.82 μM. (d) The plot of log $[(I_{F,0}-I_{F,c})/I_{F,c}]$ *vs.* log $[TiO_2]$. ($I_{F,0}$: I_F w/o TiO_2; $I_{F,c}$: I_F at TiO_2 concentration of c with I_F; NADH fluorescence intensity at 460 nm).

The presence of TiO_2 NPs in solution resulted in a decrease of NADH fluorescence intensity, most likely due to charge transfer quenching from the photoexcited NADH to the TiO_2 NP surface.[2, 3] Because the fluorescence intensity of the NADH/TiO_2 suspension was constant over time without UV irradiation, the reduction in fluorescence intensity after the addition of TiO_2 NPs could not be due to the catalytic decomposition of NADH in the dark. The Stern-Volmer plot of the NADH fluorescence intensity *vs.* TiO_2 concentration showed a linear relationship when the TiO_2 was present in the concentration range of 3.9 ~ 62.5 mg/L

(Fig. S2(c)). Negative deviations from the linear regression line were observed when the TiO_2 concentration was increased above 62.5 mg/L. Such behaviour is usually observed when the fluorescence quenching is accompanied by quencher binding.[3, 4] In this case, it is possible to deduce the binding constant of NADH to the TiO_2 surface from the intercept of the linear regression as $K_a \sim 2.5 \times 10^7$ M^{-1} according to Eqn (S1) (Fig. S2(d)).[3]

$$\log\left[\frac{I_{F,0} - I_{F,c}}{I_{F,c}}\right] = \log K_a + n\log[TiO_2] \qquad \text{(S1)}$$

S3. Dependence of the NADH photo-oxidation rate on NADH and TiO_2 concentrations

The dependence of the NADH photo-oxidation rate on the TiO_2 concentration at various NADH concentrations has been summarized in Table S2 and Fig. S3(a). The apparent rate constant, k_{app}, could be obtained from the linear regression for the relative fluorescence intensity *vs.* UV irradiation time. The k_{app} values were linearly dependent on the TiO_2 concentration in the range of 3.9 ~ 31.3 mg/L regardless of the NADH concentration in the range of 31 ~ 250 μM. When the TiO_2 concentration was lower than 3.9 mg/L, the PCA of suspended TiO_2 NP was too small to allow observation of the reaction rate with low relative uncertainty. On the other hand, when the TiO_2 concentration was higher than 31.3 mg/L, saturation behaviour was observed due to the absorption and scattering of UV light by TiO_2 NPs. To obtain consistent PCA values, the measurement should be performed under the experimental conditions that show linear dependence of k_{app} values on the TiO_2 concentration. In our experiment, k_{app} values showed linear dependence on the TiO_2 concentration when TiO_2 suspension was prepared with a transmittance between 3.2×10^{-2} and 0.72 (0.15 ~ 1.5 in optical density) at 312 nm measured using a cell with 1-cm path length.

Table S2 Photo-oxidation rate constant (k_{app})[a] of NADH in the presence of TiO_2 NPs

$[TiO_2]$ (mg/L)	[NADH] (μM)			
	31.3	62.5	125	250
1.96	0.05	0.27	0.93	1.96
2.61	0.13	0.31	1.29	2.04
3.91	0.51	0.84	1.32	2.41
5.21	1.30	1.15	1.96	3.30
7.82	1.61	2.34	2.89	4.08
15.7	3.80	4.68	6.53	7.24
31.3	6.17	7.87	11.6	13.0
62.5	8.40	11.2	13.7	17.7
125	7.92	14.2	15.1	21.0

[a] The unit of k_{app} is μM/min.

∗ pH value was adjusted to 8 with 5 mM phosphate buffer

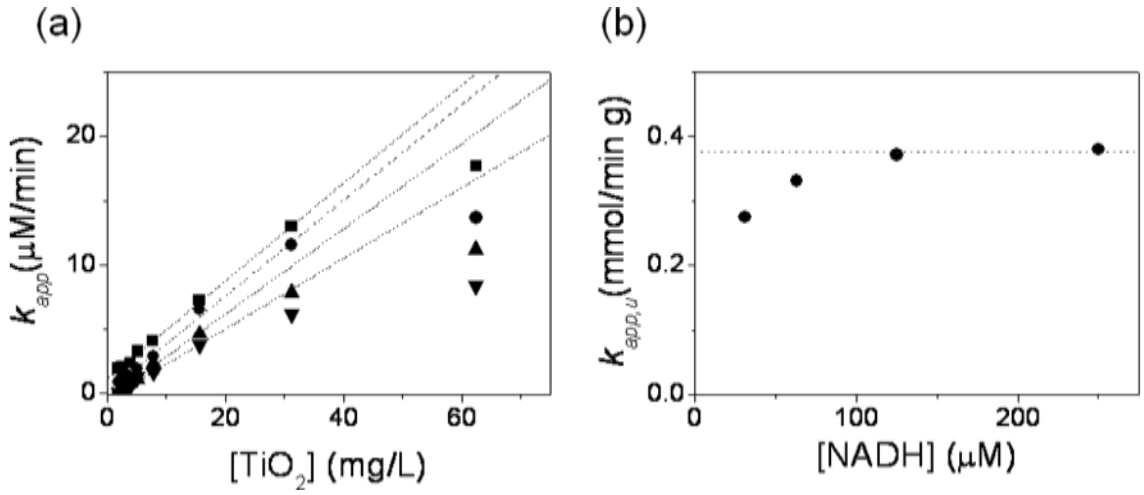

Fig. S3. (a) The dependence of k_{app} values on TiO_2 concentration. The concentration of NADH was (■) 250, (●) 125, (▲) 62.5 and (▼) 31.3 μM, respectively. (b) The dependence of the TiO_2-normalized apparent rate constant on the NADH concentration.

The slope in the linear range of k_{app} *vs*. TiO_2 concentration gives the normalized value of the apparent reaction rate constant, $k_{app,u}$, with respect to TiO_2 concentration. As shown in Fig. S3(b), the value of $k_{app,u}$ changed with the concentration of NADH.

An increase of *ca*. 35% in $k_{app,u}$ value could be observed when NADH concentration was increased from 35 to 125 μM. The variation of $k_{app,u}$ value was observed to fall within 2.5% at the concentrations of 125 and 250 μM. Thus it is preferred to perform PCA analysis

in the NADH concentration range of 125 ~ 250 μM, where consistent values of $k_{app,u}$ can be obtained to minimize the concentration effect of the substrate. From the above results, proper concentrations of the substrate and photocatalyst could be determined with regard to the linearity of the fluorescence intensity and the reaction rate.

S4. pH dependence of NADH photo-oxidation rate

It has been reported that the substrate degradation by PCA is dependent on the pH of the suspension.[5, 6] We determined the photo-oxidation rate of NADH in the presence of TiO_2 at various pH values by monitoring the reaction in pH buffered suspensions. The pH of the suspensions was varied from 5 to 11 with interval of pH 1.

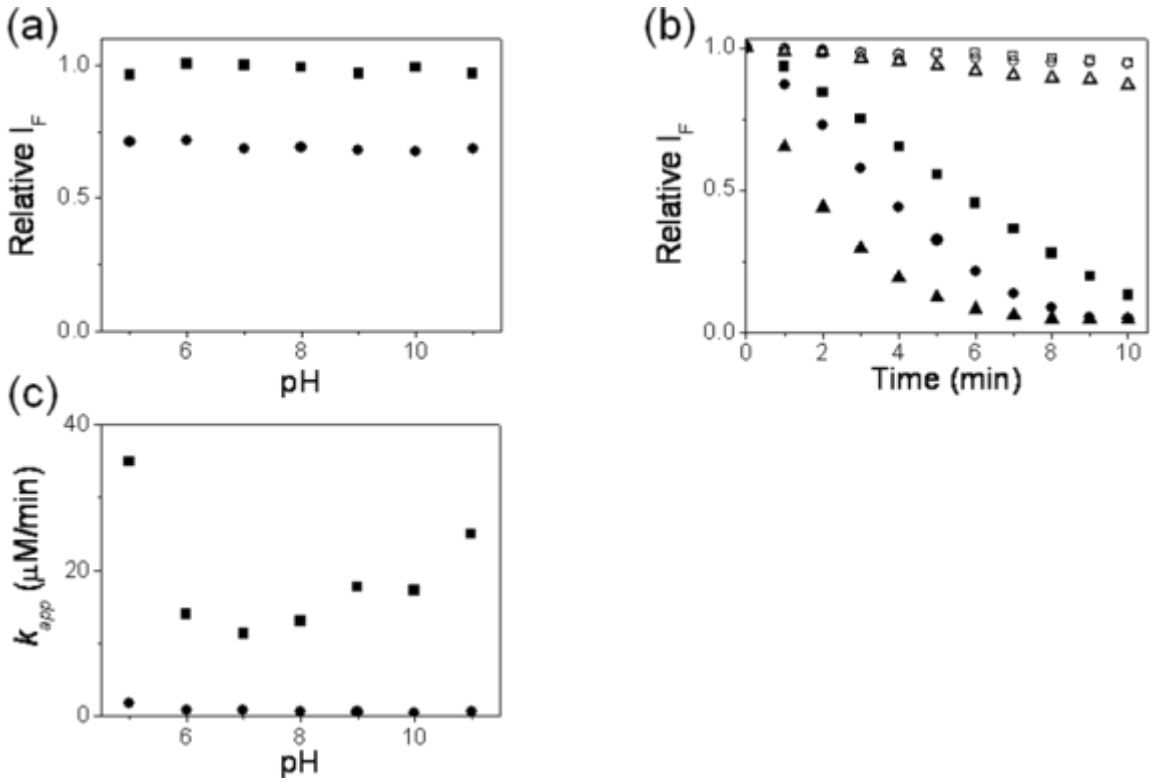

Fig. S4. (a) Relative fluorescence intensities of NADH normalized by the intensity of a pH 7 NADH solution (125 μM) in the absence (■) and presence (●) of TiO_2 NPs. (b) The decrease in the relative fluorescence intensity as a function of UV irradiation time in the absence (open symbol) and presence (closed symbols) of TiO_2 NPs at various pH values. (▵, ▲) at pH 5, (□, ■) at pH 7, and (○, ●) at pH 10. (c) The dependence of the NADH photo-oxidation rate on the suspension pH in the absence (●) and presence of (■) TiO_2 NPs.

Table S3 pH dependence of photo-oxidation rate constants

pH	Photo-oxidation rate constant[a] (k_{app})	
	NADH only	with TiO_2
5	1.70	35.0
6	0.846	14.0
7	0.754	11.3
8	0.654	13.1
9	0.500	17.7
10	0.476	17.3
11	0.648	25.0

[a]The unit of k_{app} is μM/min.

The fluorescence spectrum of NADH was not altered by the change of pH in the range of pH 5 and pH 11. The degree of NADH fluorescence quenching by TiO_2 was also similar in the pH range of interest (Fig. S4(a)). This result shows that the NADH adsorption to the TiO_2 surface was not influenced by the pH of the suspension. The photo-oxidation rate of NADH without TiO_2 in solution slightly decreased from pH 5 to pH 10 exhibiting a very low rate constant and the rate increased again at pH 11 (Table S3 and Fig. S4(b)). However, the photo-oxidation rate of NADH in the presence of TiO_2 was greatly altered by the pH change in the suspension. The k_{app} value was modulated up to 3 fold during the pH change of the suspension from pH 5 (acidic) to pH 11 (basic) (Fig. S4(c)). Therefore, it is noted that the suspension pH should be defined using pH-buffered suspensions in the PCA analysis to obtain consistent results.

S5. The dependence of NADH photo-oxidation rate on pH buffer ions

We also observed an effect of the composition or the concentration of buffer ions on the PCA measurement. The photo-oxidation rate was not altered when the composition of the buffer ions was changed, but it was dependent on the concentration of the ions. When we performed PCA analysis in acetate and phosphate buffers of pH 6, the results agreed within 0.7% (Fig. S5(a)). On the other hand, the photo-oxidation rate was modulated more than 2

fold by the change in the phosphate ion concentration at pH 8 (Fig. S5b). The reaction rate was decreased when the buffer concentration was increased from 1 to 50 mM and it increased again with an increase in the buffer concentration from 50 to 200 mM. From the above results, it is suggested that the buffer concentration and the pH of the suspension should be held in constant in the measurement of PCA using NADH photo-oxidation.

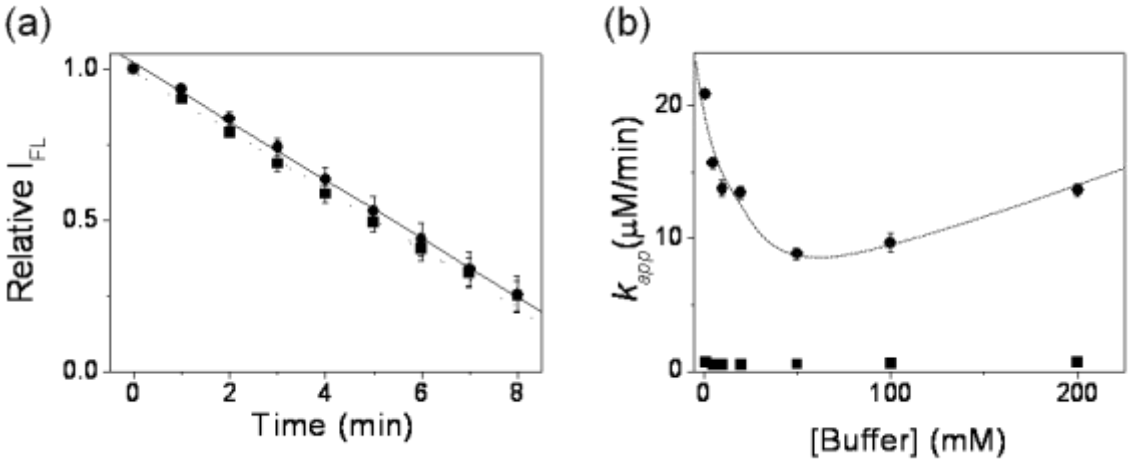

Fig. S5. (a) The decrease in the relative fluorescence intensity of NADH as a function of the UV irradiation time in the presence of TiO_2 NPs at pH 6 adjusted using acetate (■) or phosphate (●) buffer. (b) The dependence of the NADH photo-oxidation rate on the concentration of pH buffer ions in the absence (■) and presence (●) of TiO_2 NPs. (The dashed line is a simple auxiliary line for the presentation of tendency)

S6. Photo-oxidation kinetics of NADH in the presence of TiO_2 NPs

The photo-oxidation rate of NADH becomes higher in the presence of TiO_2 NPs because various kinds of radicals and ROS can be generated on the surface of the NPs. The participation of ROS generated on the TiO_2 surface in the photocatalytic oxidation of NADH could be observed through the PCA assay in the presence of ethanol, which is known to be a hydroxyl-radical scavenger.[7] The photo-oxidation rate of NADH in the presence of TiO_2 was reduced by 34 % when 2.5 mM ethanol was added (Fig. S6).

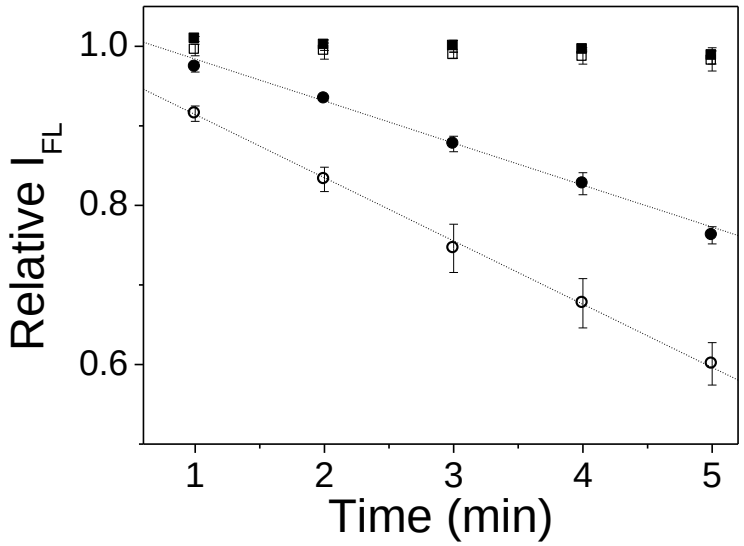

Fig. S6. The decrease in the relative fluorescence intensity as a function of UV irradiation time in the absence (open symbol) and presence (closed symbols) of 2 mM ethanol as the radical scavenger. (□, ■) 125 μM NADH solution, (○, ●) 125 μM NADH and 100 mg/L TiO_2 suspension.

When UV light is applied, holes (h^+) and electrons (e^-) are generated on the surface of TiO_2 as a result of photocatalytic activation.

$$TiO_2 + hv \longrightarrow h^+ + e^- \quad \text{(S2)}$$

Holes and electrons generate hydroxyl-radical or superoxide by reaction with water, hydroxide ion, or adsorbed molecular oxygen to oxidize NADH.[5, 8-11]

$$h^+ + H_2O_{ads} \longrightarrow \cdot OH_{ads} + H^+_{ads} \quad \text{(S3a)}$$

$$h^+ + OH^-_{ads} \longrightarrow \cdot OH_{ads} \quad \text{(S3b)}$$

$$NADH_{ads} + \cdot OH_{ads} \longrightarrow NAD\cdot_{ads} + H_2O \quad \text{(S3c)}$$

$$e^- + O_{2,ads} \longrightarrow O^-_{2,ads} \quad \text{(S4a)}$$

$$NADH_{ads} + O^-_{2,ads} + 2H^+_{ads} \longrightarrow NAD^+_{ads} + H_2O_2 \quad \text{(S4b)}$$

Here, the subscript '*ads*' indicates the species adsorbed on the surface of TiO_2 NPs.

The dependence of the NADH photo-oxidation rate on the suspension pH can be explained by the above suggested reaction mechanisms. In the suspension of pH 7, ROS are generated *via* the reactions in Eqns (S3a) and (S4a); however, ·OH generation becomes more effective in basic suspensions (pH > 7) through the reaction in Eqn (S3b). This process was expressed as an increased rate of NADH photo-oxidation along with the increase of suspension pH. On the other hand, the reaction of NADH with $O_2^{\cdot-}$ in Eqn (S4b) becomes faster in acidic suspensions (pH < 7) due to the increased concentration of the adsorbed aqueous protons (Fig. S4(c)).

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