Supplementary Material (ESI) for Chemical Communications

Electronic Supporting Information for the Article

TiO_2 Nanoparticles Labeled Kinase Activity and its Inhibitor Assay

Materials

Kemptide (H-LRRA**S**LG-OH), Protein Kinase A (PKA, Catalytic subunit from bovine heart), ATP, Bovine serum albumin (BSA), Mercaptoacetic acid (MAA), *N*-hydroxysuccinimide (NHS), *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC), were purchased from Sigma. 1-Hexadecanethiol was from Fluka (Buchs, Switzerland). Titanium dioxide nanoparticles were obtained from Degussa (P25, Germany). Acetonitrile (ACN, 99.9%) and trifluoroacetic acid (TFA, 99.8%) were purchased from Merck (Darmstadt, Germany), while ammonium bicarbonate, 2,5-dihydroxybenzoic acid (DHB, 98%), and all other chemical reagents were purchased from Sigma-Aldrich Co. Milli-Q water (18.2 MΩ-cm) was used throughout the experiments.

Preparation of a Stable Suspension of Commercial TiO2 Nanoparticles.

The P25 TiO_2 particles were heated at 300 °C for 2 h and then separated in a mortar for 2–3 h. During the separation procedure, 1mL of 10% acetic acid (in water) was added for every 1g of TiO_2 particles drop by drop to keep them wet. After being separated, the nanoparticles were suspended in an aqueous solution of ethanol (89% (v/v), TiO_2 concentration was 100 mg/mL), followed by sonication for 1 h. The resulting TiO_2 suspension could keep stable for several months. Before use, this suspension was diluted in water by 25×.

Immobilization of the substrates on electrode.

The procedure for the preparation of the modified electrode is summarized in the scheme 1. The gold electrode was polished carefully with alumina slurries (1.0, 0.3, and 0.05 mm), followed by sonication in distilled water. The electrode was then cycled between 0 and +1.5V in 0.5 mol L−1 H_2SO_4 until a stable cyclic voltammogram was obtained and then drying with purified N2. The cleaned Au electrode was then immediately immersed in a solution of 10 mM MAA in absolute ethanol at 4°C for 12 h, thus forming a self-assembling monolayer with free carboxyl groups exposed on the surface. Afterward, the electrode was thoroughly rinsed with absolute ethanol and dried with purified N2. The carboxyl groups of MAA were activated by immersing the electrode for 60 min in an aqueous solution containing 0.4M EDC and 0.1M NHS, followed by rinsing with deionized water. Then, the electrode was immersed overnight in desired concentrations Kemptide solutions using kinase assay buffer (50mM HEPES (pH 7.5) containing 0.1mM EDTA and 0.1% Triton X-100. After rinsing with blank HEPES, 100mM ethanolamine was added onto the resulting electrodes, and incubated for 30 min in a humidified chamber to deactivate and block the excess reactive succinimidyl groups remaining on the surface. The substrate-immobilized gold electrodes were rinsed with blank HEPES and stored at 4 ◦C until use.

Protein kinase A (PKA) assay on modified gold surface.

Protein kinase assay was performed on the surface-immobilized peptide substrates. Briefly, the reaction mixture contained the final assay concentration of 150 μ M ATP, 0.1mg/ml BSA, 0.2% β-mercaptoethanol and a desired amount of PKA in 500μL HEPES buffer solution (HEPES, pH 7.4). The reaction mixture (500μL) was added onto the Kemptide-immobilized gold electrode surface and incubated for 60 min at 30 ◦C in a humidified chamber. The reaction was terminated by rinsing the electrode thoroughly using blank HEPES. For the inhibition experiments, the inhibitor at a desired concentration was included in the reaction mixture. After 4 h of incubation at 30 ◦C, the reaction was terminated and the electrodes were rinsed using blank HEPES.

Electrochemical measurement on gold electrode surface.

Electrochemical DPV measurements (CHI 660c) were performed at room temperature in a 10mL electrochemical cell with a normal three-electrode configuration consists of the saturated calomel reference electrode (SCE), the platinum wire counter electrode and the working electrode after modification and silver deposition, the reference electrode was separated from the working solution by a double electrolytic salt bridge filled with saturated KNO_3 in order to avoid determination interfere caused by the continuous leaching of chloride anion that lead to AgCl precipitation. DPV determination of deposited silver was performed in 0.1mol/L HAc–NaAc buffers (pH 5.2). The DPV scan was ranged from +0.10 to +0.80V (versus SCE).

The characterization of surfaces on Au electrode.

The spectral characteristics of peptide modified electrode surfaces were investigated by FT-IR spectrometer (Nicolet company, USA) Figure-S1. shows the strong peaks at 923 $cm^{-1}$are attributed to *phosphoer*, while the peak at 1268 cm^{-1} is attributed to kemptide, respectively. We investigated the effect of immobilized kemptide concentration using DPV. We observe that the 350 μ M kemptide-immobilized electrode has the highest signal in the detection (see Figure-S4). So in the article experiment, we ues 350 μ M Kemptide as the right concentration to modify the electrodes.

Figure Captions

Figure S1. FT-IR spectra of phosphorylation-kemptide/Au modified electrode.

Figure S2. TEM images of TiO_2 mixed with $AgNO_3$ before (left image) and after UV irradiation (right image) washed with a sodium thiosulfate fixer solution.

Figure S3. Plot for the dependence of UV light irradiation time on the silver signal.

Figure S4. Plot for the dependence of immobilized kemptide concentration on the silver signal.

Figure S1.

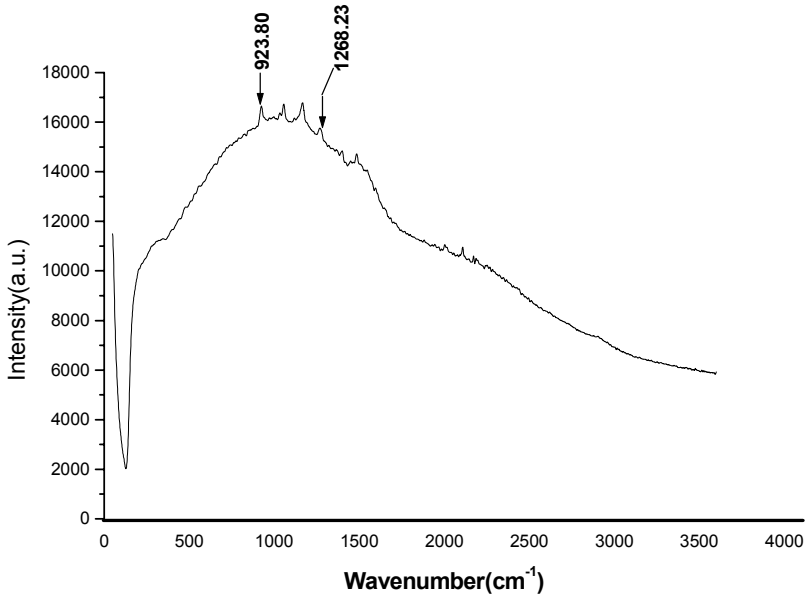

Figure S2.

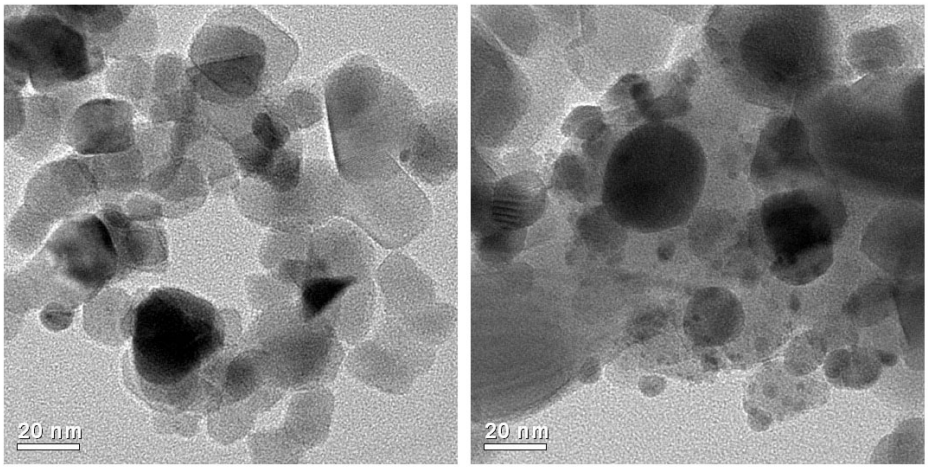

Figure S3.

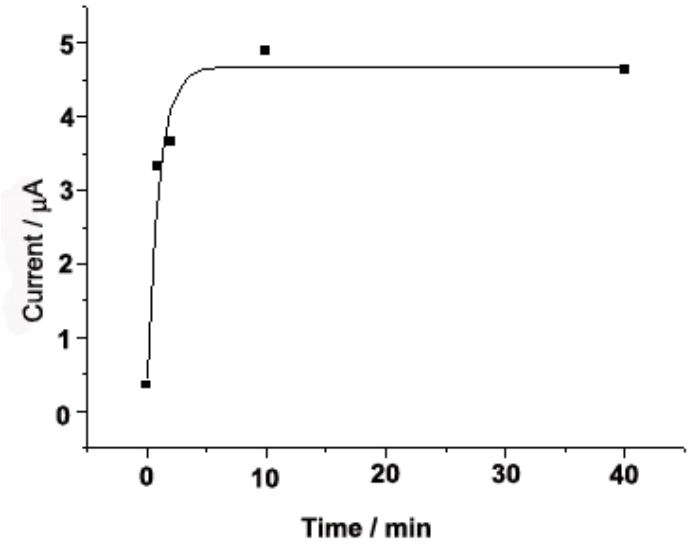

Figure S4.