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ARTICLE TYPE

Electro-catalytically active multi-protein assemblies using nanoscaled building blocks

Sven C. Feifel*, Andreas Kapp, Roland Ludwig, Lo Gorton, Fred Lisdat

Supporting information

Description of Chemicals: 11-Mercaptoundecanoic acid (MUA), 11-mercapto-1-undecanol (MU), horse heart cytochrome *c* (cyt *c*), ammonium hydroxide (99.99%), tetraethyl orthosilicate (99.99%), ethanol absolute, (3-aminopropyl)-triethoxysilane, succinic anhydride, toluene anhydrous (99.8%), DNA from calf thymus, and lactose anhydrous are purchased from Sigma-Aldrich (Steinheim, Germany), tetrahydrofuran spectranal is purchased from Riedel-de Haen (Seelze, Germany), di-potassium hydrogen phosphate, potassium di-hydrogen phosphate are provided by MERCK (Darmstadt, Germany), and gold-wire electrodes by Goodfellow (Bad Nauheim, Germany), gold chips for QCM are delivered by QSense (Frölunda, Sweden). Three different buffers have been used during these investigations: 5 mM potassium phosphate buffer pH 7, 0.5 mM potassium phosphate buffer pH 5, and 20 mM phosphate-citrate buffer pH 4.5. A fresh stock solution of β-D-(+)-lactose was prepared 1 day before experiments and stored overnight at 4 °C to reach mutarotational equilibrium. All solutions were prepared in 18 MΩ Millipore water (Eschborn, Germany).

Description of enzymes: The two forms of *Trametes villosa* cellobiose dehydrogenases (deglycosylated-CDH and native-CDH) were used, as solutions of 7.25 $g \cdot L^{-1}$(deglycosylated-CDH, 357 U/mL) and 7.0 $g \cdot L^{-1}$ (native-CDH, 154.7 U/mL) in sodium citrate buffer pH 5.5. The standard enzyme assays were done as published previously.[1] All solutions were prepared in 18 MΩ Millipore water (Eschborn, Germany).

Fabrication of nano-biomolecular assemblies – Modified electrodes: Gold-wire electrodes are cleaned by 2 times incubation in Piranha solution (3:1 H_2SO_4/H_2O_2) for 10 min (Piranha solutions must be handled with greatest caution). The electrodes are washed with Millipore water after the cleaning steps. For the construction of nano-biomolecular layers the electrodes are modified by incubation for 48 h in 5 mM 3:1 solution of mercaptoundecanol/mercaptoundecanoic acid. CDH, cyt *c* and SiNPs or DNA are immobilized by simple chemo-physical adsorption. The cyt *c* monolayers are prepared by incubation of the electrodes in 30 µM cyt *c* in KPP 5 mM pH 7 for 2 h.[2] For the nano-biomolecular assembly with SiNPs a premixed protein solution was

made in potassium phosphate buffer (5 mM, pH 7), which contained cyt *c* (20 µM) and CDH (2 µM) in a 10:1 ratio and for the biomolecular assembly with DNA a premixed protein solution was made in potassium phosphate buffer (0.5 mM, pH 5), which contained cyt *c* (20 µM) and CDH (2 µM) in a 10:1 ratio. The assembly of CDH•cyt *c*/SiNPs nano-biomolecular layers has been performed by alternating incubations of the cyt *c* monolayer electrode in CDH•cyt *c* (1:10) and SiNPs (5.0 mg•mL^{-1}) solutions for 10 min per step. Each of the 10-min long adsorption steps of SiNPs (5.0 mg•mL^{-1}) and a premixed CDH•cyt *c* (1:10) solution was followed by rinsing the electrodes with 5 mM KPP pH 7, and the assembly of CDH•cyt *c*/DNA biomolecular layers has been performed by alternating incubations of the cyt *c* monolayer electrode in CDH•cyt *c* (1:10) and DNA (0.2 mg•mL^{-1}) solutions for 10 min per step. Each of the 10-min long adsorption steps of DNA (0.2 mg•mL^{-1}) and a premixed CDH•cyt *c* (1:10) solution was followed by rinsing the electrodes with 0.5 mM KPP pH 5. The incubation procedures were repeated until the desired number of layers was reached.

QCM measurements: A Q-Sense-D E4 piezoelectric instrument (QSense, Västra Frölunda, Sweden) was used for the quartz crystal microbalance measurements. A clean gold covered quartz sensor chip (5 MHz, QSense, Västra Frölunda, Sweden) was incubated in ethanolic solution containing 5 mM MUA/MU (1:3) for 24 h, then rinsed with water and mounted into the QCM flow system. The solutions containing SiNPs or DNA, cyt *c* and CDH•cyt *c* of above given concentrations were successively pumped through the cell for 10 min with 5 min of buffer flow in between, at a flow rate of 25 µL/min. We estimated the mass increase [Δm (ng)] from the QCM frequency shift [Δf (Hz)] of the fixed films by using the Sauerbrey equation.[3] Taking into account the diameter of the electrode (d = 10 mm) and the other technical parameters,[4] this equation can be written as: $\Delta m = 1.03\Delta f$. According to this a layer mass increase can be estimated. Since measurements have been performed in solution, these values are estimated due to unknown amount of bound water.

Scanning Electron Microscopy (SEM) Measurements: For the SEM measurements, Gold-wire electrodes were modified with different supramolecular architectures (MUA/MU-cyt *c*-[DNA/dCDH•cyt *c*]$_5$ or MUA/MU-cyt *c*-[SiNP/dCDH•cyt *c*]$_5$). SEM micrographs were obtained with a Zeiss DSM 982 GEMINI (Germany) scanning electron microscope at an acceleration voltage of 3 kV.

Atomic Force Microscopy (AFM) Measurements: AFM imaging was performed in solution using a Digital JPK Instruments NanoWizard® 3 AFM (Berlin, Germany), in HyperDrive™ mode to characterize the morphology of the different supramolecular architectures on electrodes. Less damage occurs to biological specimens such as proteins when using HyperDrive™. Further details on HyperDrive™ principle can be found elsewhere.

Electrochemistry: All electrochemical measurements were carried out in a custom made 1 mL cell using an Ag/AgCl/1 M KCl reference (Biometra, Germany) and Pt-wire counter electrode. The working electrodes were modified gold wires (diameter 0.5 mm) obtained from Goodfellow (Bad Nauheim, Germany), which are modified according to the procedures described in the manuscript. Cyclic voltammetric (CV) experiments were carried out with CH Instruments CHI 660D device (Austin Texas, USA). Scan rates were varied between 0.005 and 50 V•s^{-1}, but a scan rate of 5 mV•s^{-1} was normally used to record catalytic currents, 100 mV•s^{-1} was applied for

determination of the cyt *c* concentration and to get a further impression about the limitation for the electron transfer between the cyt *c* the scan rate has been varied between 0.1 and 50 V•s^{-1}. The potential range has been chosen between -0.15 and +0.45 V *vs.* Ag/AgCl/1 M KCl. A 20 mM phosphate-citrate buffer pH 4.5 was used for all CV experiments. All measurements were performed at room temperature, 25 °C. Data analysis has been performed using CHI 660D (Austin Texas, USA) software.

Cyclic voltammograms: DNA/CDH•cyt *c* supramolecular electrode

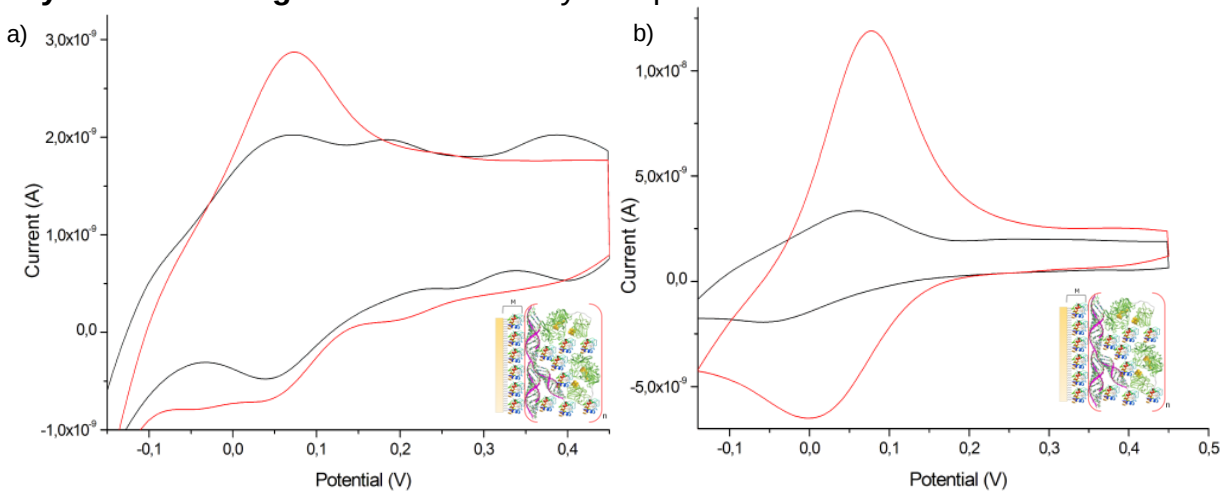


Figure 1. Cyclic voltammograms of Au-MUA/MU-cyt *c*-[DNA/CDH•cyt *c*]$_n$ multilayer electrodes with native CDH. (a) Bi-layer (n = 2) with and without lactose (5 mM). (b) Bi-layer (n = 4) with and without lactose (5 mM). A = 0.0471 cm^2, scan rate 5 mV/s, pH 4.5, 10 mM phosphate-citrate buffer.

Cyclic voltammograms: SiNPs/CDH•cyt *c* supramolecular electrodes

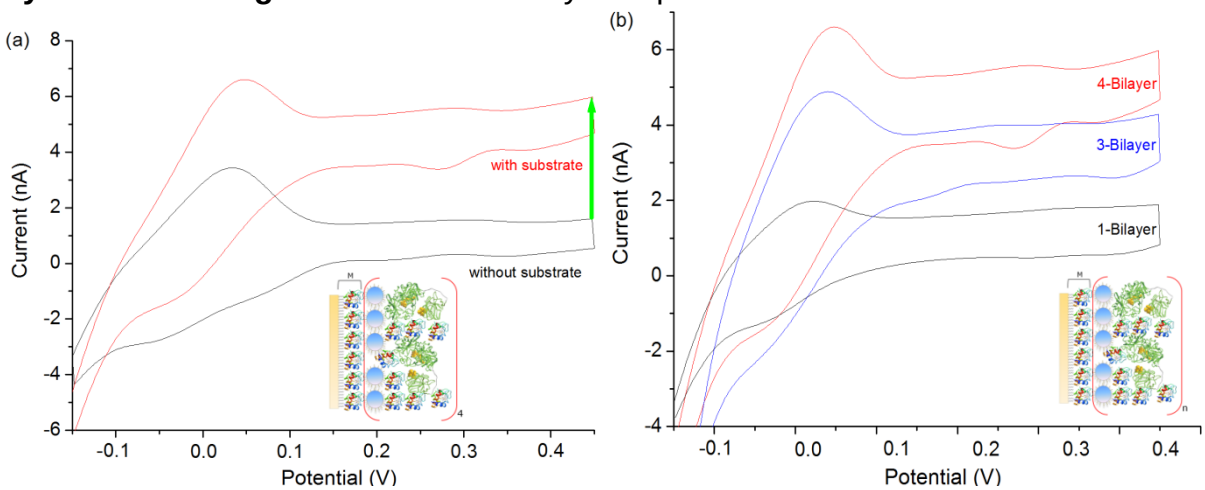


Figure 2. Cyclic voltammograms of Au-MUA/MU-cyt *c*-[SiNPs/CDH•cyt *c*]$_n$ multilayer electrodes with native CDH. (a) Multilayer (n = 4) with and without lactose. (b) Multilayer (n = 1, 3, 4) with lactose. A = 0.0471 cm^2, scan rate 5 mV/s, pH 4.5, 10 mM phosphate-citrate buffer.

Atomic Force Microscopy (AFM) Measurements: MUA/MU-cyt *c*-[DNA/dCDH•cyt *c*]$_5$ assemblies on a chip electrode

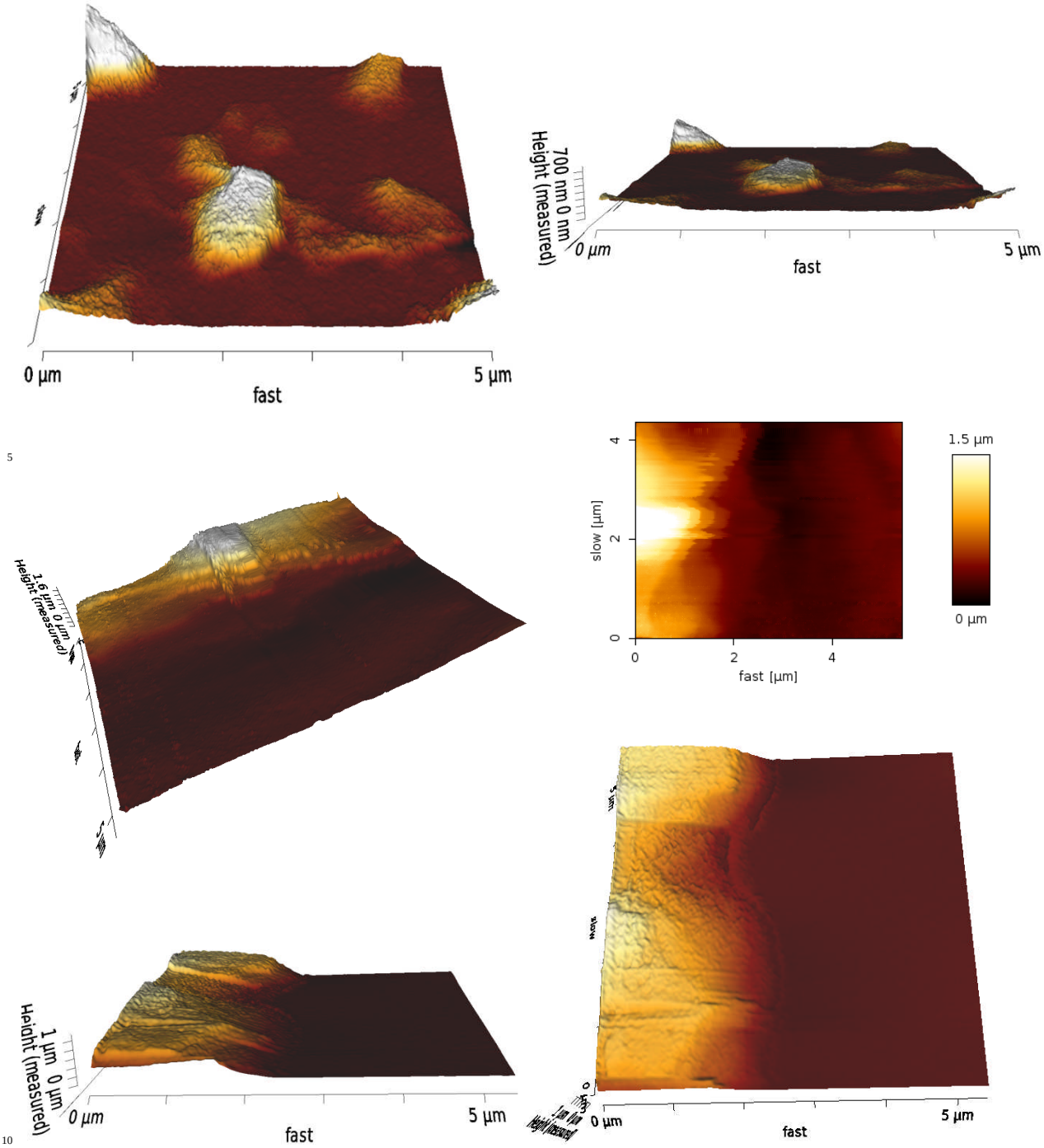


Figure 5. AFM image of a cyt *c*-[DNA-cyt *c*•dCDH]$_5$ multilayer on a MUA/MU-treated gold QCM electrode. A macula-like structure can be seen with substructures of rather large height (300 nm - 1 µm). The peak-to-valley roughness (Rt) for the DNA-based structure is about 1 µm, and the root-mean-square roughness (RMS, Rq) of the architecture is 330 nm.

Atomic Force Microscopy (AFM) Measurements: MUA/MU-cyt *c*-[SiNP/CDH•cyt *c*]$_5$ assemblies on a QCM chip electrode.

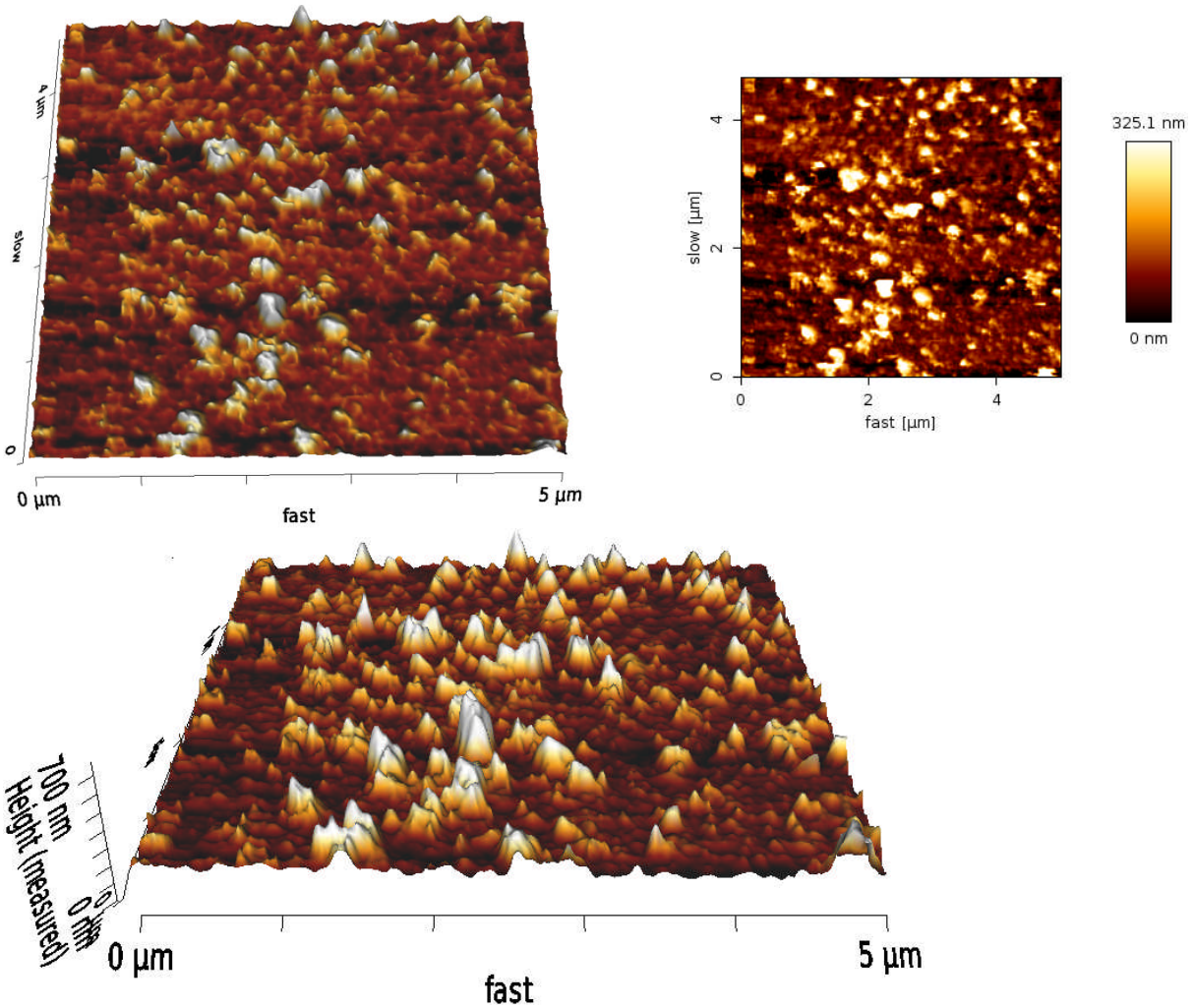


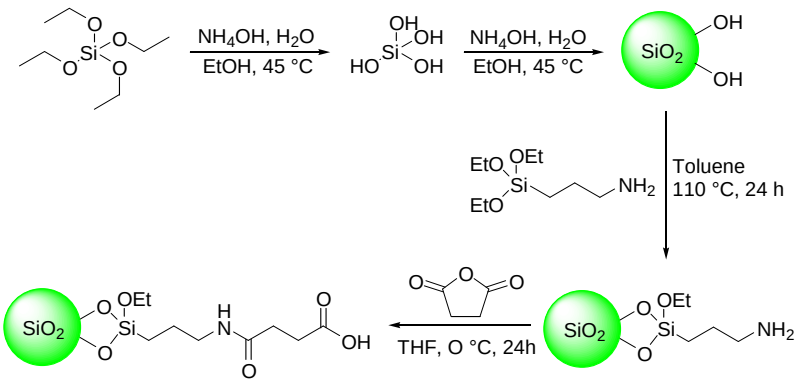
Figure 6. AFM image of a cyt *c*-[SiNP-cyt *c*•dCDH]$_5$ multilayer on a MUA/MU-treated gold QCM electrode. A hill-valley-like structure can be seen. The peak-to-valley roughness (Rt) for the SiNPs-based architecture is about 317 nm and the root-mean-square roughness (RMS, Rq) of the surface is 73 nm.

Synthesis of monodispersed SiNPs: Mono-dispersed spherical silica nanoparticles were synthesized following the Stöber-Fink-Bohn method[5] starting from tetraethyl orthosilicate (TEOS), water, ammonia, and absolute ethanol, as precursor alkoxide, hydrolyzing agent, catalyst and solvent, respectively. Two mother solutions were prepared: one containing ammonia-water and ethanol, the other containing TEOS-ethanol. The same volumes of the two solutions were always mixed in a thermostatically controlled water bath (45 ± 1 °C). A micro feed pump Harvard Apparatus (Model 11 Plus) with a constant flow rate (5.0 mL/min) fed the starting solution A (TEOS, ethanol) into the reactor to solution B (ammonia, water, ethanol) at 45 °C and vigorously stirring, thereafter the, mixture was agitated for 1 h to 5 d, dependent on the particle size to be synthesized. The SiO_2 dispersion was transferred out of the reactor and centrifuged (at 4.000-14.000 rpm for 1 h). The precipitate was washed with ethanol by repeated centrifugation (at 4.000 -14.000 rpm for 1 h) and dried at 70 °C for 20 h.

***Preparation of γ-aminopropyl modified silica nanoparticles SiO_2-NH_2*:** The amino groups modified SiO_2 particles (SiO_2-NH_2), were prepared *via* self-assembly of APTES onto the surfaces of SiO_2 particles adapted from literature.[6] First 1.0 g of SiO_2 particles were charged into a 25-mL three-necked round bottom flask containing 10 mL of dry toluene, then the suspension was dispersed with ultrasonication for 3h. Secondly, the reaction flask was equipped with an N_2 inlet, a thermometer, and a Graham condenser. Then, 2.5 mL of APTES was added quickly and the suspension was refluxed at 110 °C for 12 h under N_2 atmosphere and magnetic stirring. After the reaction finished, the suspension was centrifuged at 4.500 – 14.000 rpm for 1 h and the precipitate was collected. Finally, the precipitate was redispersed into 25 mL of dry toluene with ultrasonication for 30 min and then centrifuged again. Next, the precipitate was dispersed into anhydrous ethanol with ultrasonication and centrifuged once again. The operation of dispersion and centrifugation was repeated for three cycles, and the resulting precipitate, SiO_2-NH_2 was dried under vacuum at 40 °C for 24 h.

Introduction of the carboxyl groups onto SiO_2-NH_2 particle surface: After the amino groups were grafted onto the SiO_2 particle surface, they need to be coupled with succinc anhydride to introduce the carboxyl groups onto the surface. The coupling procedure was adapted from literature.[7] Briefly, SiO_2-NH_2 particles (1.0 g) were dissolved in anhydrous THF (50 mL) and then the suspension was dispersed with ultrasonication for 3 h. Then succinc anhydride (3.5 g) was added in two portions to the reaction suspension at 0 °C and stirred for 2 h. Afterwards the reaction mixture was stirred at room temperature for another 24 h. The remaining succinc

anhydride was hydrolyzed by addition of water. The resulting product (SiO_2-COOH) was dispersed by ultrasonication for 30 minutes and centrifuged at 4.000-14.000 rpm for 1 h. Next, the precipitate was redispersed in anhydrous THF and centrifuged again. Finally, the precipitate was dispersed into water and centrifuged for another 30 min at 4.000 rpm, and the resulting precipitate, SiO_2-COOH, was dried under vacuum at 40 °C for 24 h.



Scheme 1. Synthetic route for synthesis of silica nanoparticles. Surface modification was achieved *via* self-assembly of APTES followed by coupling reaction of succinic anhydride for introduction of the carboxylic function on SiNPs surface.

Characterization of the prepared silica particles: The synthesized SiNPs were characterized by ***dynamic light scattering analysis* (DLS)**. DLS was used to monitor the change in hydrodynamic radius (particle size) and aggregates. Measurements were carried out on a Beckman Coulter Delsa Nano C Particle Analyzer (Krefeld, Germany) working at a fixed angle of 90° in ethanol or water to obtain the number-average diameters of the particles. Each analysis was repeated at least three times to give the average particle size.

Zeta potential of the SiNPs was measured with a Beckman Coulter Delsa Nano C Zeta Potential Analyzer (Krefeld, Germany) and the measurement was repeated three times, and the average of them was reported as the final result.

Table 1. Zeta potentials.

Zeta potentials of the synthesized and modified SiNPs

SiO_2-Nanoparticles	Bare SiO_2 spheres	Modified SiO_2-NH_2 spheres	Modified SiO_2-CO_2Hspheres
Zeta Potential (mV)	-41.44	+39.63	-54.71

FT-IR analysis for monitoring the surface modification on SiNPs was measured by Fourier-transform infrared spectroscopy (FT-IR) with a Varian 680-IR FT-IR spectrometer (Varian, Australia), ATR (Diamond), resolution 8 cm^{-1}, scans 16, in the range of 400-4.000 cm^{-1}.

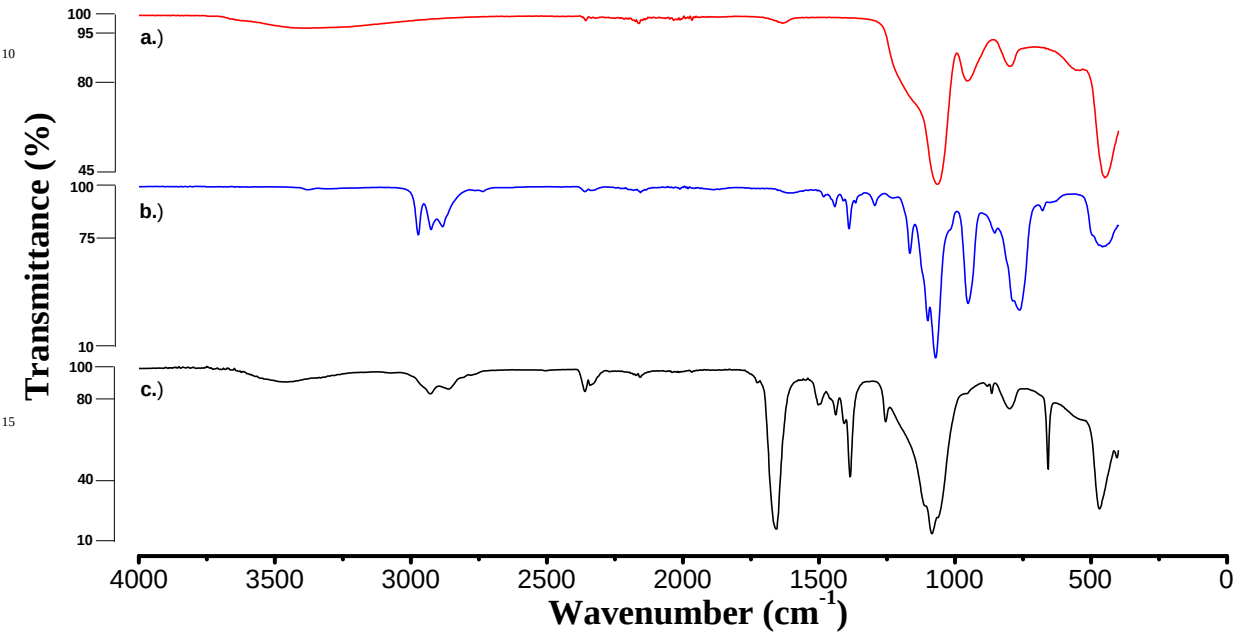


Figure 3. FT-IR spectra of the synthesized and modified silica nanoparticles. (**a**) SiO_2, (**b**) SiO_2-NH_2, (**c**) SiO_2-CO_2H. ATR (Diamond), resolution 8 cm^{-1}, scans 16, in the range of 400-4.000 cm^{-1}.

TEM measurements were applied to characterize the morphology and size of the different SiNPs, with a FEI Tecnai G^2 20 S-TWIN transmission electron microscope, 200 kV operation voltage, resolution 0.24 nm, EDAX EDX-system with a Si(Li)-detector, detection limit start at Bor (Z=5).

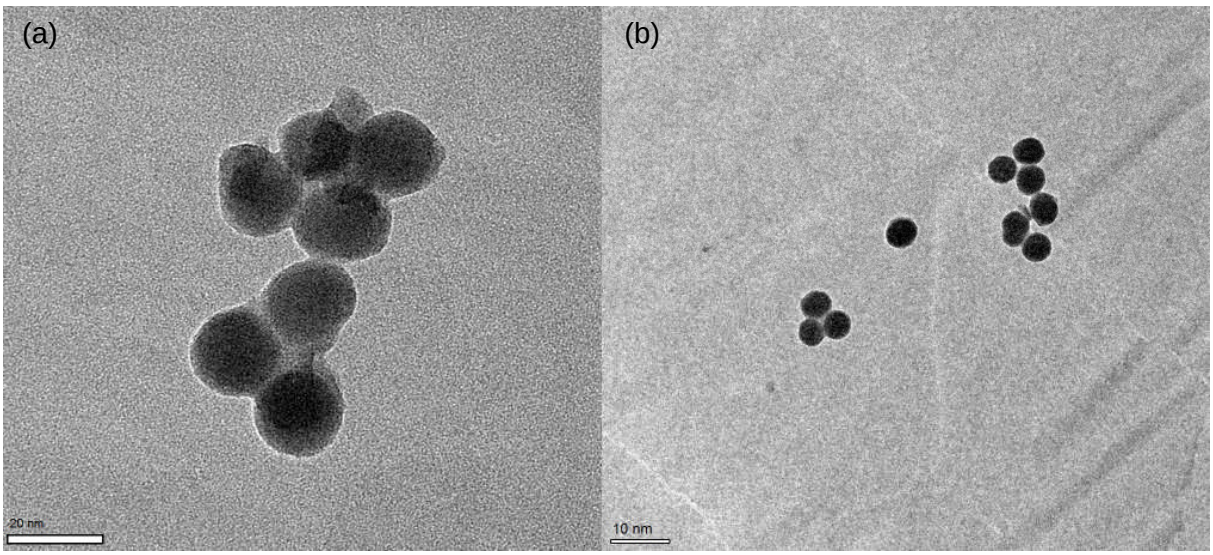


Figure 4. TEM images of synthesized silica nanoparticles.(**a**) 20 nm silica particles, (**b**) 5 nm silica particles, 200 kV operation voltage, resolution 0.24 nm, EDAX EDX-system with a Si(Li)-detector, detection limit start at Bor (Z=5).

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