

Biofunctionalization of Silica-Coated CdTe and Gold Nanocrystals

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ABSTRACT

This contribution reports the synthesis of water-soluble silica-coated CdTe nanocrystals that possess an ideally designed ligand shell with respect to colloidal properties and surface coupling reactions. We describe conjugation strategies for the modification of the fluorescent biocompatible nanocrystals with biomolecules that provide a molecular recognition potential like the biotin/avidin couple and DNA.

One of the current major challenges in materials chemistry is the synthesis of complex nanoscaled structures whose properties can be tuned by manipulating the arrangement of their constituents. Molecular recognition structures are powerful tools for organizing nanoparticles in solution. Considering the specificity and the strength of the interaction ($K_a \approx 10^{15} \text{ M}^{-1}$),¹ the biotin/avidin couple represents an extraordinary example of the molecular key–lock system in life processes.² The binding of inorganic nanoparticles to biological molecules has recently created a quickly expanding field of research that deals with numerous aspects of biosensors, medical diagnostics, and drug delivery up to fundamental investigations on the sector of self-organizing nanoelectronics.³ An example is the labeling of biological substrates with noble-metal nanoparticles or fluorescent semiconductor particles.^{4–6} To prepare such nanobioconjugates, the surface chemistry of the nanoparticles must be such that the stabilizing ligands are fixed to the nanoparticle and possess terminal functional groups that are available for biochemical coupling reactions if required. The binding to the particle surface is frequently done via thiol groups. In the case of gold nanoparticles, thiolated DNA serves as a quasi-ligand.^{3,7–12} On many materials, however, a simple thiol bond to the particle surface is not sufficient to accomplish a permanent linkage. Instead, an equilibrium will be established

with dynamic ligand exchange. To avoid this, a shell of silica is often grown on the particle itself by means of a sol–gel technique,^{13–15} and the linkage groups pointing outward are added as functionalized alkoxysilanes during the polycondensation process.^{16–18} Recently, the linkage of thiolated DNA to the surface of silanized CdSe/ZnS particles via a heterofunctional linker was presented.¹⁹

On an ideally shaped surface, the number of linkage groups should be adjustable, and inert functional groups that determine the solubility and the surface potential of the particles should be added. The linkage groups of the ligands should adhere to the crystallite surface as much as possible, and the ligands should be covalently linked together to avoid exchange completely.

In the following discussion, we describe how to synthesize such an optimized ligand shell on CdTe nanocrystals and present the coupling to biotin and the conjugative effect of the particles bound to biotin with avidin. The conjugation with DNA, carried out via phosphoramidate bonding, will be demonstrated.

The CdTe nanocrystals were coated according to a method we described previously for gold NPs.¹⁸ This method was modified with respect to the precoating with an alkoxysilane, which serves as the basic layer for the deposition of the silica shell. The schematic coating process is illustrated in Scheme 1. Whereas the gold particles were synthesized in the

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Scheme 1. Schematic Composition of the Ligand Shell

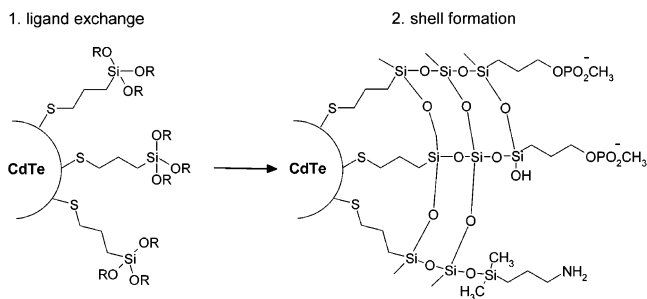
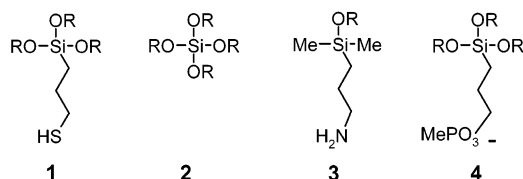


Chart 1. Structural Formulas of Silanes Used in This Work^a



^a 1: R = methyl, 2 and 3: R = ethyl, and 4: R = H.

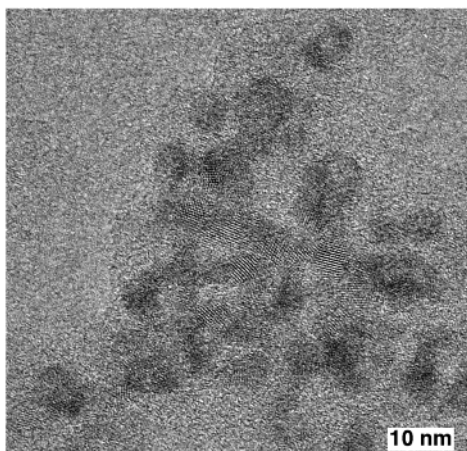
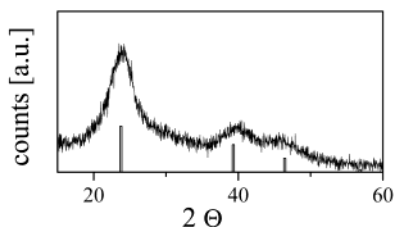


Figure 1. HRTEM image of silica-coated CdTe nanocrystals and related XRD.

presence of 3-mercaptopropyltrimethoxysilane (ligand 1), the CdTe nanocrystals were precoated by a ligand exchange with ligand 1 on preformed cysteamine-stabilized CdTe nanocrystals. On this surface, different silanes (ligands 2, 3, and 4) were condensed, leading to a cross-linked, negatively charged shell that possess outwardly directed amino groups (see Chart 1).

The resulting silica-coated CdTe nanocrystals were soluble in water and stable for a month at pH 7 to 11. Figure 1 shows the HRTEM image of the silica-coated CdTe particles. The related powder X-ray diffractogram reveals the cubic crystal structure of CdTe.

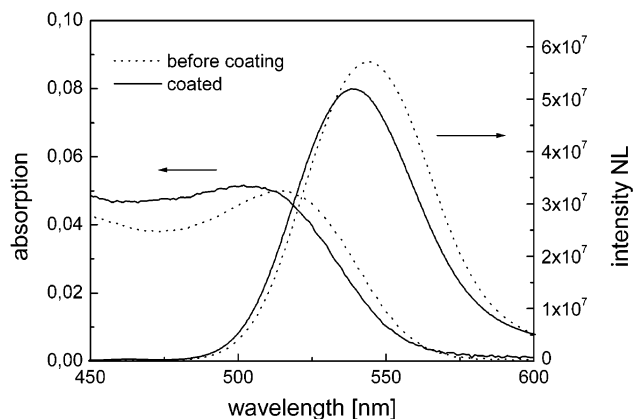


Figure 2. Absorption and luminescence spectra of the CdTe nanocrystals before and after coating.

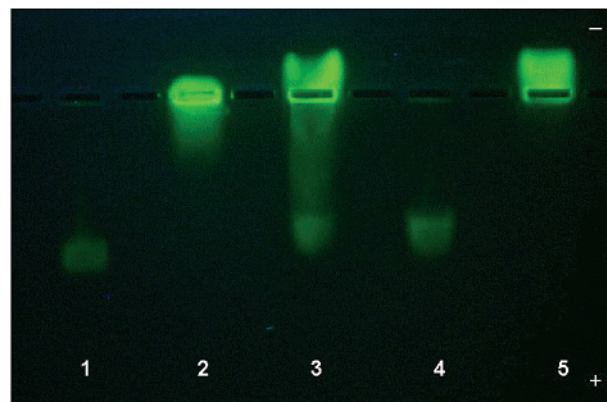


Figure 3. Luminescence image of the native gel electrophoresis of CdTe-FITC-avidin conjugates: (1) biotinylated CdTe, (2) biotinylated CdTe + FITC-avidin, (3) unbiotinylated CdTe + FITC-avidin, (4) unbiotinylated CdTe, and (5) FITC-avidin.

The absorption and luminescence spectra of the CdTe colloids before and after coating are presented in Figure 2. The spectra of the silica-coated colloids are blue shifted, which can be explained by a degradation process due to the ligand exchange.

Silica-coated CdTe nanocrystals carrying amino groups were reacted with the activated ester of the biotin.^{5,20–22} This molecule binds with outstanding selectivity and specificity with avidin proteins. The phosphonate groups on the particles cannot bind to biotin, but they provide a negative surface charge over the entire pH range and cause the desired water solubility during the biotinylation of the amino ligands. Even after the biotinylation, the colloidal and luminescence properties of the particles remained stable for months.

To prove the modification with biotin, we mixed FITC-labeled avidin with the biotinylated particles and characterized it by gel electrophoresis (Figure 3). The resulting conjugates form extensive networks since both components possess several binding sites. Accordingly, these conjugates do not show any mobility in the electrophoresis experiments (well 2). To confirm the specificity of the conjugation process, unbiotinylated particles were incubated with avidin (well 3). The mixture exhibits a superposition band at neutral pH conditions of the slightly positively charged avidin and the band of the single particles.

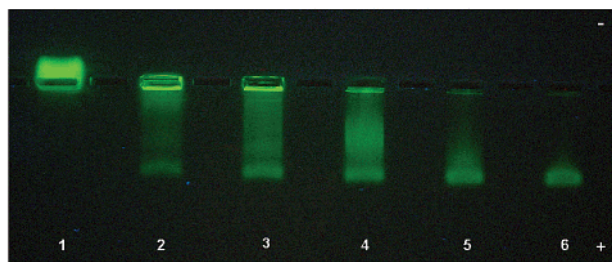
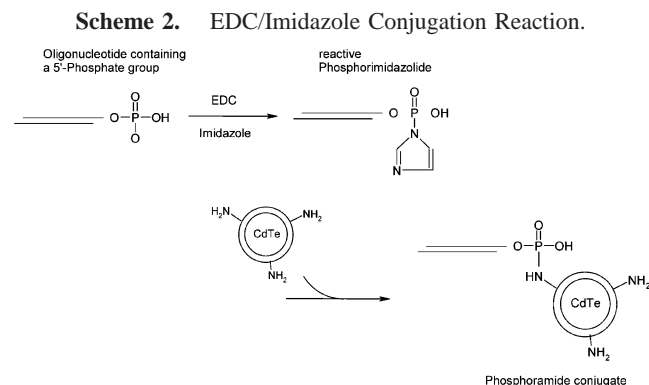


Figure 4. Luminescence image of the gel electrophoresis of conjugates consisting of biotinylated CdTe NPs and FITC-avidin with different particle/avidin ratios: (1) FITC-avidin; (2) 3:1, (3) 6:1, (4) 15:1, and (5) 75:1 biotinylated CdTe-avidin conjugates; and (6) biotinylated CdTe NPs.



With increasing biotinylation, the hydrophilic nanoparticle surface becomes more and more negatively charged, effecting a higher mobility in the gel electrophoresis (well 1) compared to that in the unbiotinylated particles (well 4).

By means of mixing one component with the other component in excess, the formation of small, discrete bioconjugates should be possible. Figure 4 shows the electrophoresis results of conjugates with different particle/avidin ratios. In a 3-fold excess of particles, the conjugates remain inside the well (well 2). An excess of unconjugated CdTe particles appears in a fraction that can be assigned to the biotinylated CdTe nanoparticles (well 6) whereas in a 6-fold or higher surplus the conjugates become more mobile toward the anode. Apparently, the avidin is surrounded by the negatively charged particles that mainly affect the overall charge of the conjugates. Because of the size of the conjugates, their mobility is reduced in comparison with that of the pure biotinylated particles (well 6). Significant AFM images of the conjugate structure were not achieved because of the limited lateral resolution of the device.

Furthermore, the silica-coated, amino-functionalized CdTe particles were reacted with DNA via a direct approach by means of phosphoramidate bonding. The reaction is illustrated in Scheme 2. The 5'-phosphate group of the DNA (250 bp DNA ladder) forms a reactive phosphorimidazolide intermediate by activation with *N*-(3-(dimethylamino)-propyl)-*N'*-ethyl-carbodiimide (EDC) and imidazole. The activated phosphate group reacts with the surface amine sites of the particles. Because of the possible reaction of the phosphonate groups on the particle surface, which would lead to a cross linking of the particle, the activated DNA was

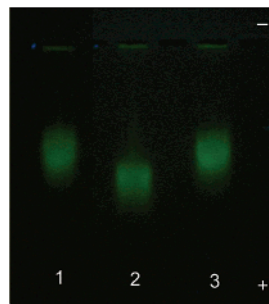


Figure 5. Luminescence image of the gel electrophoresis of CdTe-DNA conjugates that were prepared by the phosphoramidate method: (1) pure CdTe NPs, (2) CdTe-DNA conjugates, and (3) CdTe NPs + DNA without EDC activation.

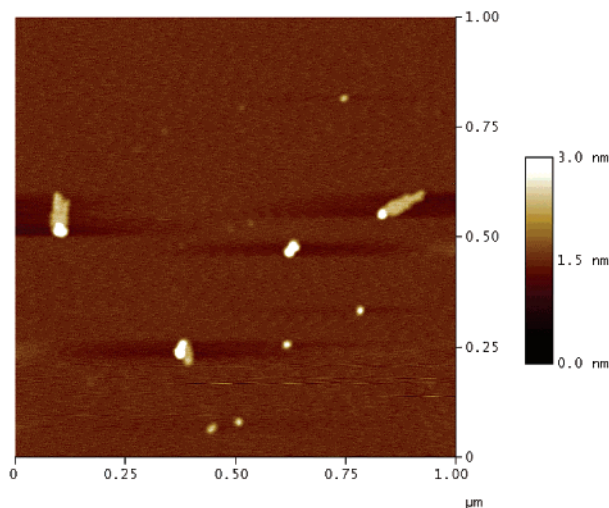


Figure 6. AFM image of the CdTe-DNA conjugates that were prepared by the phosphoramidate method after isolation from gel electrophoresis.

purified before the reaction with the particles. The particle-DNA conjugates were characterized by gel electrophoresis. The luminescence image of the native gel (Figure 5) represents the fluorescence of the CdTe particles. The conjugated CdTe nanoparticles (well 2) possess a higher mobility toward the anode than the pure particles (well 1). This effect can be explained by an increase in the negative charge due to the coupling of the negatively charged DNA to the nanocrystals. This is according to the results of Parak and co-workers¹⁹ who also observed an increased mobility of DNA-nanocrystal conjugates compared to that of the pure nanocrystals. To investigate the nonspecific adsorption of DNA to the nanocrystals, both the DNA and the nanocrystals were incubated without any activating EDC. In the gel electrophoresis, the nanocrystals of this mixture (well 3) reveal no change in the mobility versus that of the pure particles, confirming the specificity of the phosphoramidate bonding. Figure 6 shows the AFM image of the isolated fraction. A significant proportion of the particles do not appear to have DNA attached. However, one can recognize spherical particles with 100-nm-long stretched DNA strands bound to them.

We also conjugated silica-coated, amino-functionalized gold nanocrystals with thiolated oligonucleotides via the

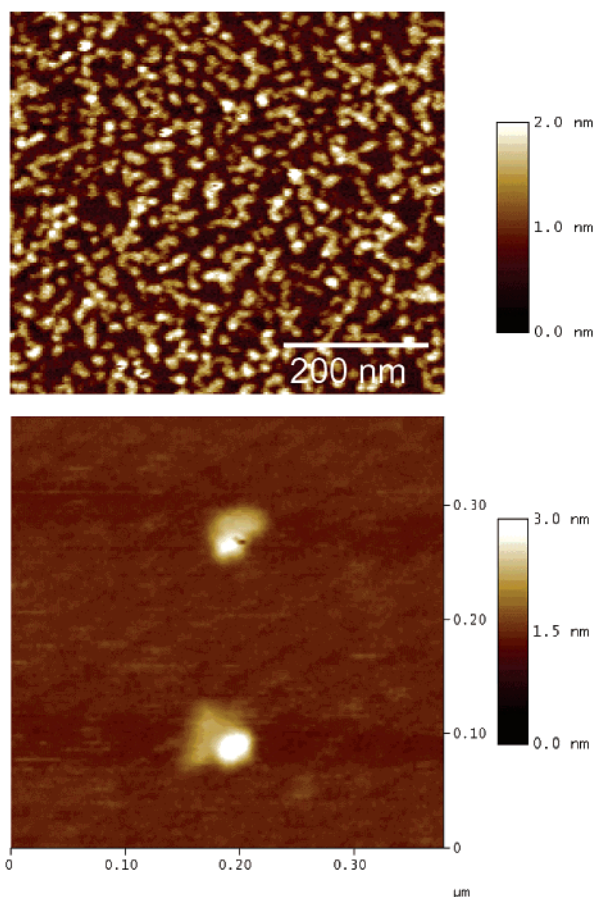


Figure 7. AFM images of the gold–DNA conjugates that were conjugated via the heterobifunctional cross linker *N*-succinimidyl bromoacetate after isolation from gel electrophoresis.

heterobifunctional linker *N*-succinimidyl bromoacetate that cross links primary amine and thiol groups. A similar method was published recently.¹⁹ The synthesis of the coated gold nanocrystals and their principal suitability for bioconjugation has been described in a previous publication.¹⁸ The AFM images in Figure 7 show gold-(100b) oligonucleotide conjugates that were purified by gel electrophoresis. The spherical particles are coupled with one or more ~30-nm-long DNA strands. Further isolation of discrete conjugates with one, two, or three strands could not be achieved with chromatographic methods such as gel electrophoresis or gel filtration chromatography.

In summary, CdTe nanocrystals have been coated with a silica shell provided with functional anchor groups. The biocompatible surface is ideally designed with respect to the conjugation with biomolecules. The luminescent particles were covalently bound to biotin, and discrete particle–avidin assemblies have been created. Before and after biotinylation, the particles that were surface-modified in such a way remained stable over months and did not show any coagulation. The silica-coated and amino-functionalized CdTe and gold nanocrystals were conjugated with DNA via different conjugation strategies. Those were first, the phosphoramidate bonding between the surface amine groups and the 5'-phosphate of the DNA and second, the cross-linker-mediated linkage of the surface amine groups and the thiol group of

thiolated oligonucleotides. The purified conjugates composed of particles with different numbers of DNA stands have been visualized by AFM.

A. Preparation of Conjugates with CdTe Nanocrystals.

A.1. Surface Functionalization of CdTe Nanocrystals. The synthesis of cysteamine-stabilized CdTe nanocrystals followed the standard technique in aqueous solution.^{23–25} The CdTe colloids of 5 mL ($c(\text{NP}) = 86 \mu\text{M}$) of intensively dialyzed solution were precipitated by the addition of 2 mL of 2-propanol and 40 μL of 1 M NaOH and were redissolved in a solution of 8 μL of 3-mercaptopropyltrimethoxysilane, 500 μL of methanol, and 10 μL of 25% methanolic tetramethylammonium hydroxide under ultrasonic treatment. The solution was successively diluted with methanol to 50 mL and refluxed for 10 min. After cooling, the solution was mixed with 100 μL of tetraethoxysilane, 2 mL of H_2O , and 1 mL of 25% methanolic tetramethylammonium hydroxide and refluxed for 30 min. Then, 225 μL of 42% trihydroxysilyl-3-propylmethylphosphonate and 50 μL of 3-aminopropyltrimethylethoxysilane were added, and the solution was refluxed for another 30 min. To silylate the active silanol groups, the solution was reacted with 26 μL of trimethylchlorosilane and refluxed for 2 min.

The transfer into water and the purification of the excess reactants were carried out 16 h later. The clear solution (6 mL) mixed with 2 mL of H_2O was reduced to 1 mL by rotary evaporation and applied to a Sephadex G-25 column that was eluted with H_2O .

A.2. Biotinylation of CdTe Nanocrystals. The purified colloidal solution (800 mL, $c(\text{NP}) = 55 \mu\text{M}$) was mixed with 200 μL of a 100 mM K_2HPO_4 buffer (pH 7). Succinimidyl 6-[biotinamido]hexanoate (1 mg) was dissolved under N_2 in 50 μL of dry DMF and added to the CdTe colloids under stirring. After 14 h, the resulting clear solution was purified by gel filtration with a Sephadex G-25 column.

FITC–Avidin–Particle Conjugates. For a series of experiments with various particle/avidin ratios, we mixed 30 μL of the purified solution of biotinylated colloids ($c(\text{NP}) = 37 \mu\text{M}$) with 15 μL of FITC-labeled avidin solutions of different concentrations (1.5 mg protein mL^{-1} (3:1); 0.75 mg protein mL^{-1} (6:1); 0.3 mg protein mL^{-1} (15:1); 60 μg protein mL^{-1} (75:1)) and shook them for 30 min.

As a blank experiment, 30 μL of the same but unbiotinylated CdTe colloid solution was mixed with 15 μL of FITC-labeled avidin (1.5 mg protein mL^{-1}) and incubated for 30 min.

The mixtures were characterized by gel electrophoresis using a 0.7% agarose gel in 10 mM K_2HPO_4 buffer by applying 100 V for 1 h.

A.3. DNA Conjugates with CdTe Nanocrystals. The DNA–CdTe conjugates were synthesized by phosphoramidate bonding between the 5'-phosphate group of double-stranded DNA and the surface amine sites on the silica-coated CdTe NPs that were described before. The method²⁶ was modified with respect to the application to the colloids.

Double-stranded DNA (10 μL , 5 μg , 250bp DNA ladder, Roche), 1.25 mg of *N*-(3-(dimethylamino)-propyl)-*N'*-ethylcarbodiimide (EDC), and 2.5 μL of a 0.2 M imidazole

solution at pH 6 (13.6 mg of imidazole, 200 μ L of 1 M HCl, 800 μ L of H₂O) were vortex mixed and spun for 5 min at 13 000 rpm. After the addition of another 10 μ L of the same imidazole solution, the reaction solution was mixed again and was purified by dialysis in a microdialysis cell for 15 min with water at pH 6.2 and for another 15 min with a 1 mM phosphate buffer (pH 7) or by gel filtration with a Sephadex G-75 spin column. Then, 20 μ L of the purified silica-coated CdTe NPs ($c(\text{NP}) = 70 \mu\text{M}$) were added and reacted for 30 min at room temperature.

The DNA–CdTe conjugates were characterized by gel electrophoresis (0.7% agarose gel in 10 mM K₂HPO₄ buffer, pH 7) and visualized by AFM.

B. DNA Conjugates with Gold Nanocrystals. The silica-coated, amino-functionalized gold nanocrystals were synthesized according to a previously reported method.¹⁸ The colloidal solution (1 mL, $c(\text{NP}) = 1.3 \mu\text{M}$) that had been intensively purified by gel filtration was mixed with 50 μ L of 100 mM K₂HPO₄ buffer (pH 7) and 2 mg of *N*-succinimidyl bromoacetate that had been freshly dissolved in 200 μ L of dry DMF. After 1 h of stirring at room temperature, the clear solution was applied to a Sephadex G-25 column. Thiolated single-stranded (100b) oligonucleotides²⁷ (7 nmol) were freshly purified via a Sephadex G-25 column and added to the modified gold colloids.

The DNA–gold conjugates were purified by electrophoresis using a 1% agarose gel in 10 mM K₂HPO₄ buffer (pH 7). The isolated fractions were characterized by AFM.²⁸

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- Synthesis of the oligonucleotide 100 up-thiol: 5'-thiolhexyl-phosphate-CTA CGT CGC TGA CTA CCT GCG TAG GTC CCT AGA TGG CTA ACT CGG TGC ATC GCT CAC TGG ATA CAT CAG TCC ATG AAT GAC TCG ATG ACT CAA TGA CTC G-3' was synthesized using an Applied Biosystems 392 DNA synthesizer. The oligonucleotide was prepared on a 200-nmol scale using polystyrene supports (LV200, Applied Biosystems) and standard phosphoramidites (Applied Biosystems). The phosphoramidite of 6-hydroxyhexyl disulfide, protected with a dimethoxytrityl group (Glen Research), was used to introduce the thiol group at the terminal 5' position. The thiol-modified oligonucleotide was deprotected with concentrated ammonia (0.05 M DTT, 16 h, 55 °C) and desalted via an NAP-10 column prior to conjugation with gold nanoparticles.
- AFM measurements: Freshly cleaved mica was treated with a 50 mM aqueous solution of MgCl₂ for 1 min and then rinsed with 10 drops of water while rotating at 5000 rpm on a spin coater. The solution of the conjugate was then applied to the substrate. After 2 min, the excess solution was removed by spinning at 5000 rpm while rinsing with 2 drops of water. Tapping-mode AFM measurements were made in air using either a MultiMode scanning probe microscope with a NanoScope IIIa controller and a NanoScope extender or a Dimension 3100 SPM system with a NanoScope IV controller (Digital Instruments, Santa Barbara, CA). Tetrahedral-tip silicon cantilevers (Olympus Optical OMCL-AC160TS series, resonance frequency 250–300 kHz) were used for all AFM measurements.

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