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Oxide Formation on Biological Nanostructures via a Structure-Directing Agent: Towards an Understanding of Precise Structural Transcription

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Abstract

Biomimetic silica formation is strongly dependent on the presence of cationic amine groups which hydrolyze organosilicate precursors and bind to silicate oligomers. Since most biological species possess anionic surfaces, the dependence on amine groups limits utilization of biotemplates for fabricating materials with specific morphologies and pore structures. Here, we report a general aminopropyltriethoxysilane (APTES) directed method for preparing hollow silica with well-defined morphologies using varying biotemplates (proteins, viruses, flagella, bacteria and fungi). Control experiments, pH evolution measurements and ^{29}Si NMR spectroscopic studies have revealed a mechanism of the assembly of APTES on bio-surfaces with subsequent nucleation and growth of silica. The APTES assembly and nuclei formation on bio-surfaces ensured precise transcription of the morphologies of biotemplates to the resulting silica. This method could be extended to the preparation of other oxides.

Introduction

With an increasing demand for specific-designed micro- and nanodevices as well as applications for photonics, catalysis, bioseparation, drug delivery, and bioreactors, the preparation of functional oxides that possess specific morphologies and designed inner pore structures have attracted a great deal of attention in materials science.¹⁻² Three functional oxides in particular which have contributed to numerous studies are silica, titania and alumina. Currently, various silica mesostructures including tubules, spheres, helicoids, and ribbons have been prepared using synthetic polymers, organogel, and surfactants³⁻¹⁰. It is generally accepted that the growth of silica on the surface of organic templates is due to electrostatic interactions with negatively charged silica precursors (prehydrolyzed $\text{Si}(\text{OR})_4$) with positively charged moieties on the template surface. The condensed precursor functions as a nuclei for the preferred polymerization of silicate at the organic-silicate interface¹¹⁻¹². The most promising approach for organizing inorganic materials for use in specific applications involving micro- and nanodevices is by borrowing from nature's repertoire. Nature provides sophisticated biological structures with well defined spatial distributions and various length scales that are responsible for unique and fascinating functions¹³. One of the most prominent examples, diatoms, can form a wide variety of micro-/nano-structured silica walls with fine features using silaffins as templates¹⁴. Compared with organic templates, however, there have only been a limited number of successful examples which

use biological systems as templates for preparing required micro- and nanosized mesosilica architectures¹⁵⁻²⁷.

In reviewing numerous successful biomimetic silica formation studies, it was commonly found that the presence of amine groups on the surfaces of biomolecules was critical for silica formation²⁸⁻²⁹. These amine groups are believed to catalyze the hydrolysis of organosilicate precursors (e.g. tetraethoxysilane (TEOS)), and subsequent polycondensation to produce a Si-O-Si net work. On the other hand, most proteins and biological species available for studies are negatively charged with an Isoelectric Point (PI) lower than 7. For example, Bovine serum albumin (BSA, gene bank ID CAA76847.1), a frequently used protein in biological research studies, is a globular protein having a pI of 4.7 at room temperature. As another example, a bacterial cell can be pictured as a microparticle (spherical or rod-like, depending on the strain) being separated from its external environment by its cell wall. The high constitution of lipopolysaccharides (for Gram negative bacteria such as *E. Coli*) or teichoic acids (for Gram positive bacteria) in the outer membrane make most bacteria negatively charged³⁰.

In addition, filamentous viruses such as *M13* bacteriophage (~880 nm long and ~7 nm wide), act as biomolecular nanowires comprised of ~3000 copies of major coat proteins encompassing ssDNA with a few copies of minor coat proteins at the tips. The ~3000 copies of major coat proteins constitute the side wall of the filamentous phage. For wild type *M13* phage, three negatively charged amino acid residues (one E and two Ds) are located in the solvent-exposed domain of each of the ~3000 copies of major coat proteins, resulting in an overall negatively charged side wall³¹. Another filamentous biological system, the flagellum, is comprised of a nano-filament attached to a bacterial cell surface which functions to assist in cell swimming. It can be pictured as a nanotube assembled from several thousand copies of protein subunits called FliC³². The solvent-exposed domain of wildtype FliC for bacteria (e.g., for *E. Coli*, gene bank ID ABI23966.1) contains negatively charged amino acids such as D and E with a PI estimated to be around 4.6.

Overall, the negatively charged surfaces described in these various biological species make it a challenge for developing simple and efficient synthesis methods for materials that can precisely transcribe morphological features of natural substances at the nanometer scale. To the best of our knowledge, a general method for preparing oxides utilizing various biological species as templates has never been demonstrated. In this study, we have demonstrated a general approach for faithfully transcribing a wide variety of biotemplates (from nanometer sized proteins to micrometer sized bacteria) to silica with various morphologies (from spheres to wires, see Supplementary Fig. S1) utilizing a systematic mechanism. In addition, biological structures have been applied for materials synthesis, but the mechanisms of materials formation have not been well understood. This study aims to disclose a general mechanism for oxide formation on biotemplates.

As illustrated in Figure 1, unlike the frequently used non-covalent imprinted sol-gel template techniques³³⁻³⁴, or biosilication processes²³, aminopropyltriethoxysilane (APTES) can be used as a “spacer” in between biotemplates and oxide precursors, serving as a structure-directing agent (SDA). Because of the presence of amine groups, APTES can be assembled on the surface of various biological templates via hydrogen bonding or electrostatic interactions.

Close contact of APTES with the biotemplate surface increases the biotemplate's nucleophilicity, thereby facilitating an attack on silicon within APTES (Fig. 1). This allows the formation of transitory Si-O bonds which then initiates the hydrolysis of APTES^{14, 35-36}. The hydrolyzed APTES may condense to form dimers, trimers, oligomers or even small

nanoparticles³⁷. The formed silica species are evenly assembled on the surface of biotemplates and act as nuclei for subsequent polycondensation of a growth agent (GA), such as TEOS, ensuring precise transcription of biotemplate morphologies to the resultant silica (see Supplementary Fig. S2).

The silica nuclei formed from hydrolysis of APTES have strong nucleophilicity³⁸, which promotes the hydrolysis of TEOS and other metal oxide precursors to form oxides such as silica and titania. The removal of biotemplates by calcination results in the production of materials with specific morphologies and pore architectures (Fig. 1). Since APTES can bond to biological surface via hydrogen bonding and/or electrostatic interactions, this approach will undoubtedly broaden the applications in biomimetic oxide synthesis and provide valuable insight into the development of micro- and nanodevices.

Experimental

Materials

Bovine serum albumin (BSA) was purchased from Sigma Aldrich and used as-received. *E. coli* strain *XL1-Blue*, *E. coli* strain *TG-1* are lab stock. Wild type *M13* bacteriophage was amplified by infecting an *E. coli* strain *XL1-Blue* in *Luria-Bertani* (LB) medium. Bacterial flagella were detached from *salmonella* bacteria by vortexing. Fungus was obtained by exposing the LB pellet to the atmosphere for 2 h and cultured at 37 °C overnight. The selected colony was transferred to the LB medium for amplification and used after centrifuging out of the growth medium and washed with water.

General procedure for the synthesis of silica and other metal oxides

For the preparation of silica, APTES was added to an aqueous solution of biotemplates (the amount used was calculated based on the biotemplate surface, generally five-fold the amount of APTES that can form a monolayer on the surface of biotemplates, in the range of the 10-25 mM). The solution was mixed with vortexing for about 1 min and kept in an ice-water bath for another 2 min. Next, TEOS with a final concentration of 50 – 100 mM was added to the solution and mixed with vortexing for another 3 min. The resulting mixture was allowed to stand at room temperature for 8 h. Generally, the solution became an opalescent gel after 15 min due to the formation of silica. The obtained silica was purified by centrifugation and washed with water twice. For the calcinations, the resulting silica was transferred to a steel boat, and heated at 120 °C for at least 12 h in a tube furnace. The temperature was then increased to 550 °C and kept at this level for 6 h in air.

Measurements

Nuclear magnetic resonance (NMR) experiments were performed on a Varian Mercury VNMRs 400 MHz spectrometer at 25 °C. Silicon NMR samples were prepared with a high concentration (10-100 mg) in D₂O because of a less natural abundance of ²⁹Si (4.7%). Proton-decoupled ²⁹Si NMR spectra were measured at 79.462 MHz using a broadband probe and all spectra were secondarily referenced to tetramethylsilane (TMS). Experiments were run for 10-100 scans with a 5 μs pulse width (45° tip angle) and 30 s delay. The pulse delay was determined to be long enough to allow complete relaxation of the nuclei. Scanning electron microscopy (SEM) samples were prepared by first dispersing the materials in deionized (DI) water and then casting them on a copper boat or silicon wafer. In the case of energy-dispersive x-ray spectroscopic (EDS) measurements, only a copper boat was used for the sample preparation. The samples were dispersed in DI water, placed on a carbon-coated TEM grid, washed and dried for one day prior to TEM imaging. The inner core sizes of the resulting silica structures are measured based on at least 100 different silica structures at different positions under TEM view.

Results and discussion

In a typical synthesis, APTES was first added to an aqueous solution of biotemplates under vortexing for about 3 min, to ensure optimal assembly of APTES onto the surface of the templates as well as the hydrolysis of APTES to form silicic acid as nuclei. Next, TEOS was added to the mixture under shaking for subsequent silica growth onto the pre-formed nuclei. Generally, white precipitates were observed in approximately 15 min. The precipitates were collected by centrifugation, washed with deionized (DI) water, dried in air and then calcined at 550 °C for 6 h to remove organic compositions.

Fig. 2 shows that varying silica structures (from nanoparticles to micro-/nano-hybrid 3-D structures) have been successfully prepared by using different biotemplates available in our lab. These biotemplates ranged in scale from nanometer sized bovine serum albumin (BSA, diameter ~ 5 nm) to tens of micrometers sized fungi. The resulting silica faithfully copied the morphologies and dimension of biotemplates, and exclusively formed on the templates with about 95% purity as can be seen from electron microscopy (EM) images. For example, TEM images (Fig. 2b) showed that the formation of hollow silica nanoparticles with a 5 ± 1 nm (in diameter) inner core and 3~5 nm silica shell resulted when BSA was used as a template. Since BSA is a globular protein (MW 66,000) with a diameter of ~5 nm³⁹, the consistency of silica inner core sizes (measured from TEM images) and the dimension of BSA suggests that the original sizes and morphologies of BSA have been faithfully transcribed to the silica structures at nanometer scales.

To the best of our knowledge, this is the smallest biotemplate that has successfully been used for the preparation of silica^{3,40}. Faithful transcription of the biotemplate morphologies to silica was also demonstrated by using fibrous templates such as wild type filamentous bacteriophage and flagella as shown in Fig. 2 c-f. The obtained silica nanotubes possessed inner pores of 6 ± 0.5 and 14 ± 0.5 nm, which are in agreement with the width of phage ($d = 6$ nm)⁴¹ and flagella ($d = 14$ nm)³², respectively.

APTES-directed silica synthesis was also successfully applied to micrometer sized biotemplates such as bacteria and fungus. Nut-like, branched-rod-like, and wire-structured silica were obtained using *Coccus (spherical bacteria)*, *E. coli* strain *TG1*, and fungus (mildew) as templates, respectively, with sizes ranging from 1 μ m to 50 μ m (Fig. 2g, h, i). TEM images were not shown since the large dimensions of the resulting silica made it difficult for electron beam to pass through the materials.

More interestingly, the precise and faithful APTES-directed silica transcription allows us to prepare 3D sophisticated silica structures. As shown in Fig. 2h, by using *E. Coli* with *pili* as templates, 3-D artificial silica architectures that possessed connected micrometer sized tanks and nanometer sized tubes with micro-/nano-hybrid length scales were obtained by our simple and efficient APTES-directed method (see Supplementary Fig. S3). Furthermore, the universal application of APTES-directed approach was confirmed by using biological systems with positive net charge ($PI > 7$) as templates. For instance, positively charged biological species such as engineered bacteriophage (*M13* phage displaying a positively charged peptide *R4* on the side walls) and cationic liposome were successfully used as templates to direct the growth of silica nanofibers and hollow silica spheres (Fig. 3).

Silica obtained from our APTES-directing method showed nice control of morphologies, sizes and pore structures which are otherwise difficult to control through conventional synthesis. Thus, it would be more promising if we can convert the synthesized silica to silicon with retained morphologies and pore structures as market for silicon based devices is vast for potential applications in photonics and sensors. We investigated the possibility of conversion of the synthesized silica to silicon according to the Sandhage magnesiothermic

reduction process⁴². As shown in the supporting information (Fig. S4), pure porous silicon with retained morphologies were obtained and their application for gas sensor device is now under investigation in our lab.

To have a better understanding of silica nucleation and the growth mechanism, several controlled experiments were carried out. First, TEOS was added to the bacteria solution without APTES. No silica formation was observed after 24 h incubation at room temperature. However, addition of 1% of ammonia to the TEOS/bacteria mixture immediately leads to the formation of silica. SEM images showed the formation of silica nanoparticles with a larger size distribution (see Supplementary Fig. S5a) and no silica growth on the bacteria were observed.

The structure-directing function of APTES was also confirmed by control experiments with different addition sequence of APTES and TEOS to biotemplates. As we described above, exclusive formation of silica rods was observed when APTES was first added and then followed by addition of TEOS to bacteria *E. Coli* (with 95% purity, see Supplementary Fig. S5b). However, a reverse addition order (TEOS then APTES) or a simultaneous addition of APTES and TEOS significantly decreases the exclusivity of the formation of silica on the biotemplates. SEM images show the formation of silica rods with rough surface; together with a large amount of free silica nanoparticles in the resulting materials (Fig. S5). This was attributed to the simultaneous hydrolysis of APTES and polycondensation of TEOS, both in solution and on the surface of biotemplates. Furthermore, the addition of APTES analog, propyltriethoxysilane (PTES) (see Supplementary Fig. S6), shows no silica precipitates even after several days after mixing PTES and TEOS with bacteria in an aqueous solution. This is because PTES cannot be assembled onto the surface of biotemplates as that of APTES due to the absence of amine groups.

In a different control experiment, only APTES was added to the biotemplate solution omitting TEOS. We note that no white precipitates were formed over a period of several days. This result might indicate that APTES can only form oligomeric silicic acid as nuclei to initiate the polycondensation of TEOS (as will be discussed in ²⁹Si NMR study). APTES itself cannot polycondense to form large silica networks as that of TEOS because the polycondensation of APTES may be stopped by its propyl amine arm. Since APTES cannot form large aggregates of silica, they can assemble evenly on the biotemplate surface and function as nuclei to initiate the steady hydrolysis and polycondensation of TEOS. We believe that it is the even assembly of APTES and thus the silica nuclei it forms on the templates surface that ensures the precise transcription of biotemplate morphologies to the resulting silica structures.

The nucleation and growth of silica with the addition of APTES and TEOS to the biotemplates were monitored by the change of solution pH value as a function of time. As shown in Fig. 4, the addition of APTES to the biological template solution (pH = 7.4) changed its pH value to 11.4 due to the basic properties of APTES itself. The pH value decreased rapidly in about 2 min to around 10.5, which is attributed to the hydrolysis of APTES to form polysilicic acid as nuclei on the surface of templates⁴³. In this pH range (pH = 11.4-10.5), predominant hydrogen bonding interaction between APTES and biological surface is anticipated^{44,45} since the amine group in APTES is in its neutral form ($pK_a = 10.6$)⁴⁶. It may also give a clue about why APTES-directed method can be applied to most biological systems. In the APTES hydrolysis pH range (pH = 10.5-11.4), most biological systems (either negatively charged (PI<7) or positively charged (PI>7)) would have the similar surface properties such as with similar surface functional groups ($-NH_2$ and $-COO^-$)⁴⁷.

When the hydrolysis of APTES was slowed down, the addition of TEOS induced another rapid drop of pH value, indicating the hydrolysis and condensation of TEOS proceeding on the surface of templates. Since there are almost no free silica nanoparticles (< 5%) formed in solution, the hydrolysis and polycondensation of TEOS is believed to occur exclusively from the pre-formed APTES-directed nuclei which were pre-organized on the surface of templates. However, if the reaction was carried out in phosphate buffer solution with constant pH, not in the way with spontaneous pH drop as we described above, the faithful transcription cannot be achieved. Our control experiments (see Supplementary Fig. S7) show bacteria are shrunk and broken when the reaction was carried out in a strong base buffer solution (pH = 10-11), which was due to the denaturation of proteins. It will take several days to form aggregated silica in a weak base buffer solution (pH = 8-9) with irregular surface. These results revealed the advantage of spontaneous pH drop, which avoids the denaturation of protein through the rapid decrease of pH value through APTES hydrolysis, and also catalyzes the polycondensation of TEOS in a moderate base solution.

Because the chemical shift of silicon is determined by the chemical nature of its neighbors, namely, the number of siloxane bridges attached to a silicon atom, ^{29}Si NMR provided a convenient way to monitor the intermediates formed during the hydrolysis and condensation reactions of silicon alkoxides. Various silicon units can be distinguished by their degree of hydrolysis (i.e., the number of OH groups attached) and degree of condensation (i.e., the number of Si-O-Si bridge bonds) in ^{29}Si NMR spectra. According to references of Marsmann *et al.* and the commonly used notations⁴⁸⁻⁵⁰, M, D, T, and Q structures were used, which are corresponding to one, two, three, and four Si-O-Si bridges, respectively. For instance, T_j^j corresponds to $\text{R-Si}(\text{-OSi})_i\text{-OR}'_{(3-i)}$, where the subscript (i) indicates the number of connected -O-Si bridge bonds (i.e. the degree of condensation) and the superscript (j) indicates the number of OH groups attached to silicon atom (i.e. the degree of hydrolysis).

Pure APTES without hydrolysis showed only one peak at -44.4 ppm (see Supplementary Fig. S8). In deuterium water (D_2O), hydrolysis of APTES to form “silanols” was observed as shown in Fig. 5a, where four peaks appear at -43.8, -42.6, -41.5, and -40.4 ppm, which were assigned to T_0^0 , T_0^1 , T_0^2 , T_0^3 of silicon, respectively (see Supplementary Fig. S9). No other peaks were observed except a broad peak around -110 ppm that came from the NMR tube, suggesting that the hydrolysis of APTES to “silanols” in D_2O was a main process.

However, when APTES was added to a biotemplate solution (*M13* bacteriophage was used as an example because of its uniform and suitable size for NMR measurement), rapid hydrolysis and condensation of APTES was observed. As shown in Figure 5, after 2 min of mixing the APTES and phage solution, the unreacted APTES peak disappeared (only T_0^1 can be seen at -42.7 ppm), revealing three new broad peaks appeared at -50.7 (weak), -58.3, and -67.2 ppm, which were assigned to T_1^1 , T_2^1 , and T_3^1 , respectively.

Continuing incubation of the above mixture at room temperature for 30 min resulted in a further decrease of intensity of T_0^1 and corresponding increase of the intensities of T_2^1 and T_3^1 (Fig. 5). However, no new peaks appeared in the Q region, indicating that no tetra Si-O net works were formed from the propyl amine arm in APTES, which is consistency with the pH study.

The broadening of the peaks T_2^1 and T_3^1 can be attributed to the presence of the various hydrolyzed states of each structure (change of j value) and the unsymmetrical rotation of the molecules containing silicon atoms. When TEOS was added to the APTES/bacteriophage mixture, major silicon species became Q-structures with three broad peaks corresponding to Q2 (≈ -92.8 ppm), Q3 (≈ -103.9 ppm), and Q4 (≈ -109.8 ppm) (Figure 4), indicative of a

polymerized silica network in the resulting materials. These observations derived from ^{29}Si NMR spectra clearly show the formation of oligomeric silicic acids through the hydrolysis and condensation of APTES on the surface of biotemplates. These formed silicic acids function as nuclei to initiate and catalyze the hydrolysis and polycondensation of TEOS to form a silica network.

Since hydrolysis of silica precursor TEOS was initiated and catalyzed by the silicic acids from APTES hydrolysis, it would greatly broaden the APTES-directed method if the silicic acids from APTES hydrolysis are able to activate the hydrolysis and polycondensation of other metal alkoxide precursors. Here, titania (TiO_2) was chosen as an example. Similar to the protocol for APTES-directed silica synthesis, APTES was first added to *E. Coli* solution under stirring. After 3 min, titanium(IV) bis-(ammonium lactato)-dihydroxide ($\text{Ti}[\text{BALDH}]$) solution (50 mM) was added and white precipitates were formed in several minutes. The precipitates were collected and purified by centrifugation, characterized by SEM and TEM. Both SEM and TEM images show the faithful copying of *E. Coli* morphologies into the resulting materials (see Supplementary Fig. S10 and Fig. S11). The elemental composition determined by EDS analysis showed the presence of titanium, indicating the formation of titania. Although the pristine obtained titania are amorphous (Fig. S11), they were easily changed to anatase crystal structure through calcinations at 550 °C. Electron diffraction of the calcined titania exhibited a strong diffraction ring corresponding to a d spacing of 3.5 Å, which matched the crystal plane (101) with the highest intensity for nanocrystalline TiO_2 (anatase phase, JCPDS 84-1286).

The structure-directing function of APTES in titania growth was confirmed simply by a control experiment. In the control experiment, mixing of bacteria and $\text{Ti}[\text{BALDH}]$ without APTES showed no precipitates even after several days of incubation. These experiments further confirmed our proposed mechanism for the APTES-directed method. That is, the silica nuclei formed from hydrolysis of APTES have strong nucleophilicity, which can promote the hydrolysis of TEOS or $\text{Ti}[\text{BALDH}]$ to form silica or titania, respectively.

Conclusions

In summary, we have presented a general and efficient APTES-directed oxide synthesis on biological surfaces via a systemic mechanism study. Based on the control experiments, pH evolution and ^{29}Si NMR spectra results, we concluded that APTES was first assembled onto the surface of biotemplates as a structure directing agent through hydrogen bonding and/or electrostatic interactions. The close contact of APTES with biotemplate surfaces facilitated the nucleophilic attack of surface proteins, leading to the hydrolysis of APTES to form oligomeric silicic acids, which were evenly assembled on the surface of biotemplates and functioned as the nuclei. These nuclei imitated the polycondensation of TEOS at the chemical and biological interface forming the resulting silica framework. As a result of even assembly of nuclei on the surface of biotemplates, the morphologies of biotemplates were faithfully imparted to the resulting oxides. Various silica morphologies at varying length scales have been successfully demonstrated in this work ranging from nano-sized particles to micro-/nano-sized 3-D sophisticated structures. This method was similarly employed for the synthesis of other metal oxides such as titania. The removal of biotemplates by calcinations with retained morphology could enable the fabrication of low-cost micro- and nanodivices with specific shapes and pore structures that are otherwise difficult to produce by conventional techniques.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Schematic illustration of nucleation and growth of silica on the surface of biological templates by an APTES-directed approach. In this strategy, APTES (yellow color) was first assembled onto the surface of biotemplates through hydrogen bonding or electrostatic interactions (a), which was hydrolyzed by the surface protein to form tiny silica nuclei (yellow particles in b). Subsequent addition of a growth agent (TEOS, blue color) leads to the polycondensation of silica from the formed nuclei (c) at the chemistry and biology interface, resulting in growth of the silica particles. These growing silica particles finally met each other and fused to form a silica shell on the surface of the biotemplates (d). Calcination can be employed to remove the organic compositions which leads to the formation of hollow silica structures.

Figure 2.

SEM and TEM images of silica prepared by using different biotemplates, ranging from nanometer to micrometer scales. (a) and (b), SEM and TEM images of silica spheres prepared using BSA as templates. (c) and (d), SEM and TEM images of silica fibers prepared using wild type bacteriophages (*M13*) as templates. (e) and (f), SEM and TEM images of the necklace like silica fibers prepared using wild type flagella as templates. (g) (h) and (i), SEM images of the microscale spheres or rods prepared using *Coccus*, fimbriated *E. coli* strain *TG1*, and fungus (mildew) as templates, respectively. The inset shows the corresponding high magnification of microscopy images.

Figure 3. SEM and TEM images of silica prepared by using biotemplates with positively charged surface. (a) and (b), SEM and TEM images of silica fibers prepared by using engineered bacteriophages (*R4-M13*) as templates. (c) and (d), SEM and TEM images of silica spheres and membrane prepared by using cationic liposome (DOTAP-DOPC).

Figure 4. pH values of biotemplate (*M13* bacteriophages was used here as an example) solutions after addition of APTES and TEOS as a function of time (in seconds).

Figure 5.

^{29}Si NMR spectra study of APTES-directed silica growth. (a) Pure APTES in D_2O for 15 mins. (b) ^{29}Si NMR spectra of APTES in D_2O in the presence of *M13* bacteriophage after 2 min mixing. (c) ^{29}Si NMR spectra of APTES in D_2O in the presence of *M13* bacteriophage after 30 min mixing. (d) ^{29}Si NMR spectra of APTES and TEOS in the presence of *M13* bacteriophage, TEOS was added 5 min after the addition APTES to the bacteriophage solution and ^{29}Si NMR was collected 10 min after the addition of TEOS. Broad band centered at -110 ppm resulted from silicon in the quartz NMR tubes.