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Quantification and Stability Determination of Surface Amine Groups on Silica Nanoparticles Using Solution NMR

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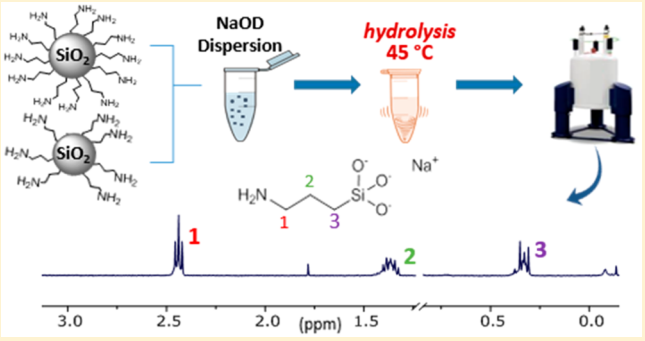
Supporting Information

ABSTRACT: Surface chemistry is a critical factor for determining the behavior of a nanomaterial after incorporation in composites, devices, and biomedical products, and is also important for nanotoxicology studies. We have developed an optimized protocol for dissolution of aminated silicas and determination of functional-group contents by quantitative ^{1}H NMR (qNMR) analysis of the released amines. A number of variables were optimized for the dissolution protocol, including the base concentration, mass of silica, time, temperature, and method of sample agitation, in order to achieve adequate NMR signals for quantification. The protocol was tested using nanoparticles from a single commercial supplier with sizes ranging from 20 to 120 nm that were functionalized with 3-aminopropyl groups. Interestingly the batch-to-batch variability for some sizes of these aminated silicas was as high as 50%. Amine contents measured by a ninhydrin colorimetric assay were typically ∼20% lower than those measured by qNMR, consistent with measurement of only ninhydrin-reagent accessible amines. The dissolution−qNMR protocol was compatible with aminated silicas from other commercial suppliers, and in these cases, an even larger variability in surface coverage was observed. Silica nanoparticles with longer-chain amines and variable amine loadings were synthesized to demonstrate the ability to quantify amines with more complex structures and to assess the limit of quantification for the dissolution−qNMR method. Finally, the stability of the aminated nanoparticles was examined. Loss of 3-aminopropyl groups occurred in water at room temperature and was significantly more rapid at higher temperatures. Amine loss increased with increasing surface coverage and was slower for long-chain amines, consistent with studies of amine stability on planar silica. Overall, this work highlights the importance of developing methods for quantifying surface functionalization, particularly given the variability in surface coverage for commercial samples, and for ensuring that the amine group is stable under its usage conditions.



Silica nanoparticles have been widely used for a range of applications, including biosensing and imaging, drug delivery, environmental remediation, and catalysis.[1−5] Both mesoporous silica, which has a large pore volume, and less porous nanoparticles (NPs) produced by the sol−gel process have been employed, and the addition of silica shells as a protective coating for other nanomaterials is also widespread. These materials have a number of useful properties, including ease of synthesis, the ability for surface modifications to change the material properties or impart targeting functionality, biocompatibility, and low toxicity. Silica NPs typically yield stable colloidal suspensions, although the stability is dependent on the surface chemistry.

Despite the extensive use of nanomaterials in a broad range of application sectors, the lack of adequate characterization has been frequently cited as a confounding factor that makes it difficult to compare results between laboratories.[6,7] A well-characterized material is essential to maintain batch-to-batch reproducibility in the production of a material, to ensure reproducible behavior for applications, and to assess potential toxicity. Controlling the surface chemistry is of crucial importance because the surface of the nanomaterial is the main point of the interaction with the external environment and will ultimately determine the fate of the material when incorporated in devices, used for biological applications, or released to the environment. A number of methods are available to identify and quantify surface functional groups. Nevertheless, the various methods have different sensitivities, limitations, and ranges of applicability, and it is still relatively rare that multiple methods and validated protocols are employed.[8−10]

Here, we report the optimization of a quantitative solution ^{1}H NMR method to measure amine coverage on the surface of silica NPs. Amines were selected as an initial target because they are one of the most common functional groups for silica.[11] They are frequently formed by reaction of amino-alkylalkoxysilanes with silanols on the surface of preformed

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silica nanoparticles. Although widely used, the production of aminated silicas presents challenges. These include polymerization of the initial silane in solution, the possibility of forming heterogeneous multilayers on the surface in the presence of excess silane, and the lack of stability of the aminated surfaces.[12−14] Hydrolysis is particularly problematic with short aminoalkyl silanes such as aminopropyl triethoxysilane (APTES), which have been shown to undergo self-catalyzed amine-mediated cleavage.[15] The self-catalyzed hydrolysis can be reduced or eliminated by the choice of more bulky or longer-chain amines.[13,15,16]

A variety of methods have been employed to quantify organic surface groups on nanomaterials.[17] Thermogravimetric analysis is one of the more commonly applied techniques, but it is complicated by the necessity of applying corrections for the concurrent loss of variable amounts of surface or pore-adsorbed water (or other solvents) for silicas.[18] Although quantitative ^{19}F NMR (^{19}F qNMR) in solution and the solid state is both selective and sensitive,[19,20] employing other heteronuclei, such as ^{31}P and ^{13}C, to achieve the necessary spectral resolution, especially in the solid state, leads to limited sensitivity and is time-consuming.[9,21] Furthermore, all of the above qNMR approaches require specially labeled functional groups. Methods based on relaxometry of solvent protons generally rely on additional methods such as specific-surface-area assumptions and modeling of the solvent−surface interactions to estimate the amount of surface groups.[22] X-ray photoelectron spectroscopy and fluorescence assays have limited sensitivity and are subject to solvent- and self-quenching problems, respectively.[23−26] A number of studies have concluded that acid−base titrations and colorimetric assays are useful for amines and carboxylic acids,[27,28] but overall there is no generally approved method for a range of materials and functional groups. Two recent reports suggest that quantitative ^{1}H NMR (^{1}H qNMR) in solution, after the particles are dissolved in strong base, may be a promising alternative for quantification of amines on silica NPs.[29,30] In related work, solution NMR has been shown to be a useful approach for measuring the dynamic equilibrium between surface-bound and solution amines for some functionalized mesoporous silicas.[31] Here we explore the advantages and limitations of the qNMR method and identify the variables that must be controlled for an optimized protocol for the dissolution of the silica NPs for amine quantification. The method is used to assess amine contents for silica NPs sourced from several commercial suppliers as well as those synthesized in house, with a focus on assessing protocol suitability for silicas prepared by different synthetic routes and with structurally different amines. The silica-dissolution−qNMR method is compared to a ninhydrin assay, which in principle measures only amines that are accessible to reagents in solution. Finally qNMR is used to study the hydrolytic stability of aminated silicas.

RESULTS AND DISCUSSION

Silica Nanoparticles. The initial measurements were conducted on commercial 20−120 nm amine-functionalized silica NPs (NanoComposix) that had been prepared by the Stöber process and functionalized with the 3-aminopropyl group. The materials were supplied as dry powders or ethanol suspensions. The supplier provided physical-characterization data, including the *z*-average from dynamic light scattering, the mean diameter from transmission electron microscopy, and the surface charge as measured by the ζ-potential (Supporting Information, Table S1). In-house-functionalized silica NPs were prepared from commercial 100 nm bare silica NPs (NanoComposix) and various aminated silanes in order to provide NPs with variable amine coverage and with longer-chain amines. Several aminated silicas from other commercial sources were also analyzed.

Solution ^{1}H NMR of Intact NPs. Prior to dissolution experiments, intact NPs suspended in a deuterated solvent were examined by NMR. The smallest size (20 nm) was selected, assuming that the more rapid Brownian motion of smaller NPs could provide sufficient mobility to average out dipolar and other anisotropic magnetic interactions. The NPs supplied in ethanol dispersion were isolated by centrifugation followed by vacuum-drying and then redispersed in EtOD-d_6 and D_2O for ^{1}H NMR. No signals corresponding to protons of the propyl amine were observed in EtOD-d_6 (Figure 1A).

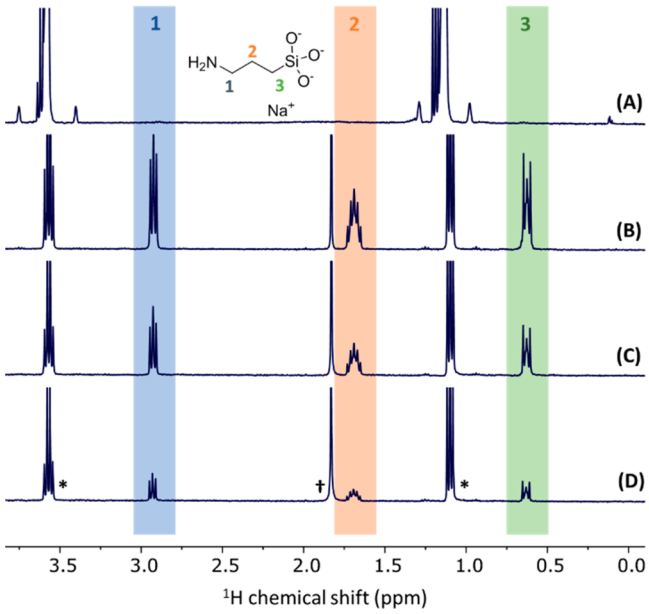

Figure 1. ^{1}H NMR spectra acquired from 20 nm $SiO_2C_3NH_2$ NPs dispersed in (A) EtOD-d_6 or (B−D) D_2O. After redispersion of the dry NPs, the samples were equilibrated for (D) 10, (C) 70, or (A,B) 100 min and then heated to 70 °C for 30 min. All NMR measurements were recorded at room temperature. Residual EtOH resonances and an unknown impurity are denoted by asterisks (*) and a dagger (†), respectively.

Interestingly, when the NPs were redispersed in aqueous medium, small signals due to propyl amine were observed (Figure 1B−D). The signals increased with time over a period of 2 h and further increased in intensity when the sample was heated at 70 °C for 30 min and then cooled to room temperature to record the ^{1}H NMR spectrum (Figures 1D and S1). By contrast, similar increases in time and heating for the sample in EtOD-d_6 provided no evidence of free amine in solution. These results suggest that the observed signals in D_2O are due to free amines in solution.

The above results indicate that hydrolysis occurs for the aminated 20 nm NPs in D_2O but not, within the limits of NMR detection, in EtOH. A similar experiment using 50 nm silica NPs demonstrated that very small signals due to amine were detected 20 min after dispersion of the sample in D_2O. The amount of amine increased with time, accounting for

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approximately 8% of the initial amine content after 2.3 h and continuing to increase over a period of 2 days at room temperature. The loss of amine from silica NPs is consistent with previous studies of ∼50 nm mesoporous silica NPs functionalized with APTES where small amounts of hydrolyzed amine were identified with the help of DOSY and NOESY NMR and quantified by qNMR.[31] A previous study in which ∼100 nm silica NPs were studied by solution NMR reported only weak and broad signals from intact NPs prior to dissolution.[30] However, these signals most likely arise from hydrolyzed amino propyl groups, as discussed above.

Optimization of the Dissolution−^{1}H NMR Quantification Method. Because the only NMR signals observed from intact NPs are due to hydrolyzed amino propyl groups, NPs were dissolved in basic media to release the surface-immobilized functional moieties into solution for quantitation by ^{1}H NMR. Earlier studies using a similar approach had employed different hydrolysis conditions, either sonication of NPs in NaOD/D_2O solution at pH 13 for 1 h or incubation in 0.2 M NaOD/D_2O at 37 °C for 16 h.[29,30]

Several different hydrolysis conditions were tested to determine the optimal hydrolysis method, including different concentrations of NaOD, variable times and temperatures, different NP sizes, and agitation by either sonication or shaking. Initial experiments using sonication of silica NPs in 0.2 M NaOD resulted in the expected characteristic ^{1}H NMR signals due to the aminopropyl moiety (Figure 2A). Note that

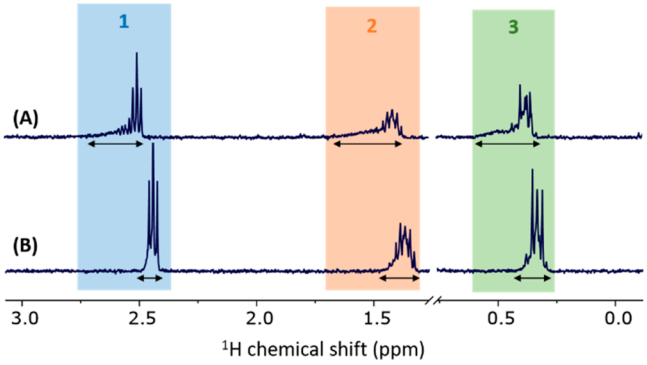

Figure 2. ^{1}H NMR spectra of 3-aminopropyl siloxane generated by dissolution of 80 nm SiO_2@C_3NH_2 NPs (batch B2) by (A) sonication in 0.2 M NaOD for 1 h or (B) heating and mixing in 0.4 M NaOD at 45 °C for 3 h. The average of the integrals for the three characteristic resonances at 0.32, 1.36, and 2.44 ppm is compared to that of the internal standard (maleic acid) at 5.89 ppm. The residual EtOH resonance at 1.09 ppm was removed for clarity.

the chemical shift of the methylene groups decreases in more strongly basic solutions (pH ∼13, Figure 2) compared with those of the samples shown in Figure 1B−D (pH ∼9). A decrease in scattering intensity as measured by both dynamic-light-scattering and UV−visible-absorption measurements (Figure S2) also indicated dissolution of the NPs. The amine content estimated from the average integral for the three ^{1}H NMR signals relative to that of the internal calibrant, maleic acid, for multiple replicates of the three sizes of NPs is summarized in Figure 3A. The variation between replicates and the broadening of the NMR signals suggested that the initial conditions were not adequate for complete dissolution of the silica, with possible formation of oligomeric species that result in broadening of the NMR resonances. Consistent with this

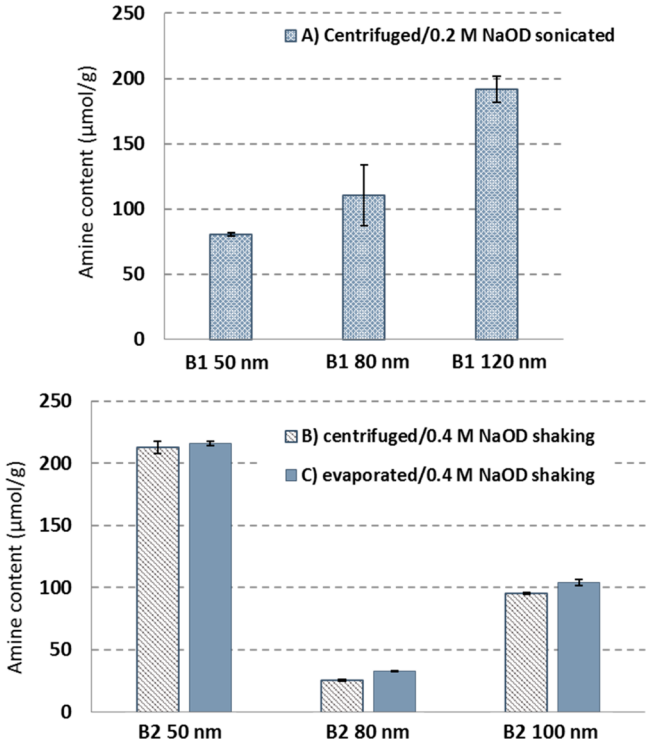

Figure 3. Amine-functional-group contents for various sizes of two different batches (B1 and B2) of $SiO_2C_3NH_2$ using different conditions for NP dissolution: (A) 0.2 M NaOD in an ultrasonic bath for 1 h and (B,C) 0.4 M NaOD in a heated mixer at 45 °C for 3 h. NPs were isolated by centrifugation (A,B) and solvent evaporation (C).

hypothesis, modification of the conditions to dissolve NPs in 0.4 M NaOD in a heated mixer at 45 °C resulted in improved NMR-signal quality, which facilitated quantification (Figure 2B), and improved reproducibility between replicates (Figure 3B). Note that these experiments were carried out on different batches of NPs because of the limited amount of materials. Interestingly the two batches of 50 nm silicas had amine contents that differed by a factor of ∼3. Comparison of single experiments for silica NPs in 0.4 M NaOD at 45 °C gave estimates of amine contents that were higher (10−20%) than the average value obtained for sonication in 0.2 M NaOD. Further experiments with increased base concentration (0.8 M) or prolonged dissolution at higher temperature did not lead to any further increase in measured amine content.

The above optimization experiments used NPs received as dispersions. NPs were exchanged into D_2O by centrifugation to pellet the sample followed by removal of the supernatant and redispersion of the pellet (see the Experimental Section). This method may lead to loss of NPs either because of incomplete pelleting of the NPs (especially for smaller sizes such as 20 nm) or because of loss during removal of the supernatant. To test whether this contributed to variability in results, the NPs were isolated by evaporating the solvent in a stream of air and then drying the NPs under vacuum. This approach resulted in a modest increase in amine content (Figure 3C) relative to the centrifugation method for some samples, suggesting that pelleting the NPs and removing the supernatant results in some loss of material and should be avoided for accurate quantification.

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Initial silica-dissolution experiments used ~10 mg of silica NPs in a fixed volume (0.65 mL) of NaOD/D_2O. The protocol was repeated with increasing amounts of silica to test whether it was feasible to completely dissolve a larger mass of silica, which would facilitate detection of lower amine loadings. The standard conditions using 12.5 mg of 100 nm silica NPs and 0.4 M NaOD produced well-defined signals for all three methylene groups. Doubling the mass of silica resulted in broadened resonances, and tripling the mass led to broad signals that were shifted toward higher chemical shifts compared with the typical propylamine resonances (Figure 4). Both of these factors potentially complicate the integration,

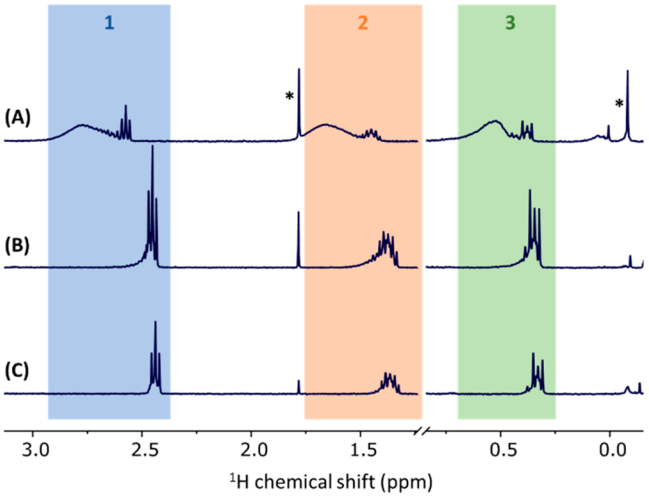

Figure 4. ^{1}H NMR spectra demonstrating the impact of incomplete dissolution of silica NPs: (A) 37.5, (B) 25, and (C) 12.5 mg of 100 nm SiO_2@C_3NH_2 in 0.4 M NaOD. The residual EtOH signal was removed for clarity. Signals denoted by asterisks (*) correspond to unidentified impurities for this batch of NPs.

making quantification less reliable. The broad signals are attributable to either incompletely dissolved silica matrix with attached aminopropyl groups or to APTES polymers. We have observed broad signals from APTES in D_2O, and similar broad signals are reported in the literature and assigned to polymers.[32] For comparison, the NMR results for 0.2 and 0.1 M NaOD are shown in Figure S3. In both cases, doubling the silica mass gives broad signals, and for 0.1 M NaOD the broad signals are even observed with 12.5 mg of silica.

Figure S3 also provides data for the integration of signals for the various combinations of silica mass and NaOD concentration. Despite the broad signals for higher silica masses, similar amine contents are measured for all three samples using 0.4 M NaOD. However, the amount of amine is underestimated by 15−50% for most of the other samples (Figure S3). These observations may indicate that variation of the base concentration and silica mass results in species with different molecular-weight distributions. Although preliminary results suggest that this does not always interfere with quantification, it appears advisable to work with silica/base ratios that avoid the appearance of broad signals.

The above experiments indicate that a mass ≤12.5 mg (in 0.65 mL solvent) should be used for the dissolution−qNMR determination of amine content. Under the conditions used for qNMR, we estimate a quantification limit of 0.2 μmol/mL in order to obtain a signal/noise ratio of ~10 for 32 transients and reliable integration of the amine signals. This corresponds to a sample with ~10 μmol/g silica as the lower limit for amine content that can reliably be determined by qNMR. Further improvement could potentially be attained via dissolving larger amounts of silica using more concentrated base, although increased sample viscosity might increase the relaxation times. Alternatively, the limit of quantification could also be decreased by using more powerful NMR spectrometers or by significantly increasing measurement time.

Amine Quantification for Different Sizes and Batches of Silica NPs. The optimized dissolution method and qNMR were used to measure the amine contents for five different sizes (20, 50, 80, 100, and 120 nm) of aminated silica NPs obtained as ethanol dispersions from NanoComposix, with three replicates for each NP size. As shown in Table 1, the amine

Table 1. Amine-Functional-Group Quantification for 20−120 nm NanoComposix Silica NPs As Measured by the Dissolution−qNMR Protocol and the Ninhydrin Assay and Compared to Estimated Monolayer Coverages[a]

SiO_2@C_3NH_2	qNMR (μmol/g)	ninhydrin (μmol/g)	estimated monolayer coverage (μmol/g)
B3 20 nm	655 ± 3	320 ± 20	920
B3 50 nm	64 ± 1	47 ± 1	360
B3 80 nm	145 ± 3	103 ± 4	230
B3 100 nm	186 ± 1	153 ± 13	180
B3 120 nm	179 ± 3	117 ± 4	150

[a]The monolayer coverages are estimated from the nominal diameter and a silica density of 1.96 m^2/g and by assuming a density of four 3-aminopropyl siloxane moieties per square nanometer. All measurements are the average of three independent replicates.

content does not show the expected trend with NP size. Assuming full monolayer coverage on the surface of the NP, smaller NPs are expected to have larger amine contents (mol/g) by virtue of their larger surface-area/mass ratios. Although the smallest-size NPs (20 nm) do have the largest amine content, there is no obvious trend for the larger NPs. For the smaller NPs (up to 80 nm in size) the amine content is lower than predicted for monolayer coverage on the basis of the surface area estimated from the nominal diameter and assuming a surface density of four 3-aminopropyl siloxane moieties per square nanometer.[33] The values for the 100 and 120 nm NPs are similar to or slightly higher than the estimated monolayer coverage, respectively. A similar trend is evident for the batches of 50 and 80 nm NPs shown in Figure 3. Note that the estimated surface areas are in reasonable agreement with the measured specific surface areas measured by the Brunauer−Emmett−Teller method for selected samples (Table S1).

The qNMR values for amine contents were compared to those measured using a ninhydrin colorimetric assay, which has been frequently used to quantify amines on planar silicon dioxide surfaces and in a few cases on silica nanoparticles.[27,28] Here we optimized several parameters, as outlined in the Experimental Section. The ninhydrin assay provides an assessment of the content of accessible amines, which may differ from the total amine content measured after dissolution of the silica NPs. The data (Table 1) demonstrate that the ninhydrin assay measures a lower amine content than the qNMR dissolution method for each size of silica NPs. The ninhydrin assay estimates are lower than those from qNMR by 20−30%, except for those of the 20 nm NPs, for which the

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ninhydrin assay accounts for only 50% of the amines measured by qNMR. This indicates that the majority of the amines are located on the surface of the NPs and thus accessible to the ninhydrin reagent. Nevertheless, the systematically lower ninhydrin values suggest that some amines are not accessible to the reagent, possibly due to crowding on the surface at close to monolayer coverages, surface multilayers, or locations in interior pores of the microporous silica.[34] By contrast, qNMR reports on the total amine contents based on dissolution of the NP. These results are consistent with other studies in which the accessible-functional-group content as measured by surface derivatization was considerably lower than the total content.[9,10] Note that the 100 and 120 nm NPs have amine contents that are close to or slightly higher than the estimated monolayer coverage. Unfortunately the ninhydrin assay is not compatible with strongly basic conditions, which prevents verification that qNMR and ninhydrin give the same result for dissolved silica NPs.

Figure 5 provides a summary of the amine contents for multiple batches of five different sizes of silica NPs. The

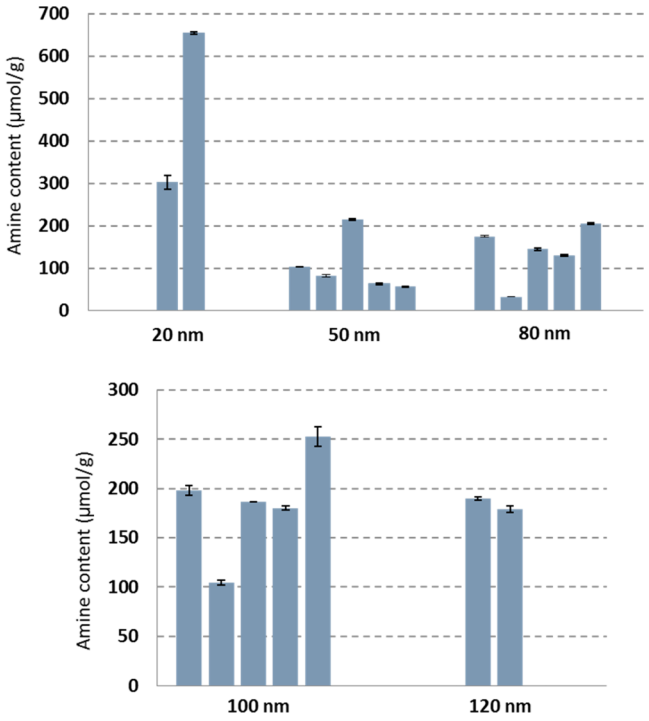

Figure 5. Determinations of 3-aminopropyl-functional-group contents in multiple batches of commercial 20−120 nm silica NPs. The data were obtained using the optimized dissolution protocol and either standard or qNMR methods.

various batches were purchased from the same supplier over a period of ∼1 year, and each batch had a different lot number. The data were all obtained using the optimized dissolution method and a combination of either the standard or qNMR protocol (see the Experimental Section); therefore, small variations (∼5%) may reflect NMR-method variability. There is at least one batch that is significantly different from the others for each of the 50, 80, and 100 nm NPs, presumably reflecting differences in the synthetic procedures for those samples. Except for the 100 and 120 nm NPs, the measured amine content is always lower than that estimated for monolayer coverage (see Table 1). These results highlight the importance of reliable methods to quantify the functional-group contents for applications in which the surface chemistry determines the fate of the material.

Measurement of Amine Contents for Silica NPs from Different Sources. Several amine-functionalized silicas were prepared in-house from a single batch of 100 nm bare NPs using different initial APTES/NP ratios. The highest amount of APTES was 1.1 times the estimated value for full monolayer coverage, assuming 100% functionalization efficiency and a monolayer density of four aminopropyl moieties per square nanometer. The surface-modification procedure, described elsewhere,[30] was conducted on an analytical scale in D_2O and EtOD-d_6, which allowed NMR quantification of both the fraction of amines attached to the NPs and the nonreacted moieties in solution. Analysis of the supernatant and the NPs indicated that 46% of the initial APTES was covalently attached to the NP surface (Table S2, 87 ± 1 μmol/g). This is approximately 50% of the estimated monolayer coverage for 100 nm diameter NPs and is lower than most of the commercial 100 nm NPs shown in Figure 5. This suggests that a larger excess of amine is used to functionalize the commercial NPs. Amine functionalization was further supported by TEM characterization (Table S3 and Figure S4) which indicated that the diameter was similar to that of the bare particles and there was no evidence of aggregation. Amine functionalization resulted in a large positive ζ-potential of +33.8 mV, compared with a value of −27 mV for the bare silica NPs. An increase in hydrodynamic diameter (≈15 nm) was measured by DLS, which may have been due to an increase in the hydration sphere caused by hydrogen bonding of the amine groups.

The amination reaction was repeated on a preparative scale using different amounts of APTES (1.1, 0.5, 0.2, and 0.1 times the estimated monolayer coverage). Interestingly, for smaller ratios of APTES (0.2 and 0.1) the reaction was almost quantitative (Figure 6). Note that the sample functionalized with the lowest amount of APTES had an amine content that was close to the estimated quantification limit of 10 μmol/g for qNMR using 32 transients.

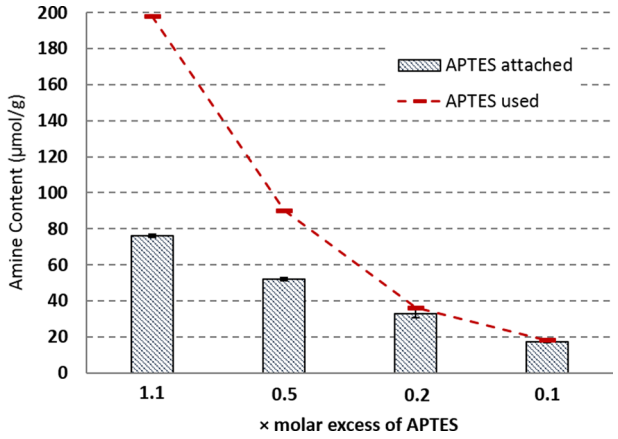

Figure 6. Surface density achieved by reaction of APTES with bare 100 nm silica NPs using different initial amine concentrations. The red points and line show the amount of amine used for surface modification. The estimated monolayer coverage is 180 μmol/g. Only the surface amine content was measured by the standard ^{1}H NMR method.

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Two longer-chain amines, (6-aminohexyl)aminomethyl and *N*-(2-aminoethyl)-11-aminoundecyl triethoxysilanes, were grafted to 100 nm bare silica NPs under similar conditions using ratios estimated for monolayer coverage. The ^{1}H NMR spectra of the hydrolyzed NPs are displayed in Figure 7. For

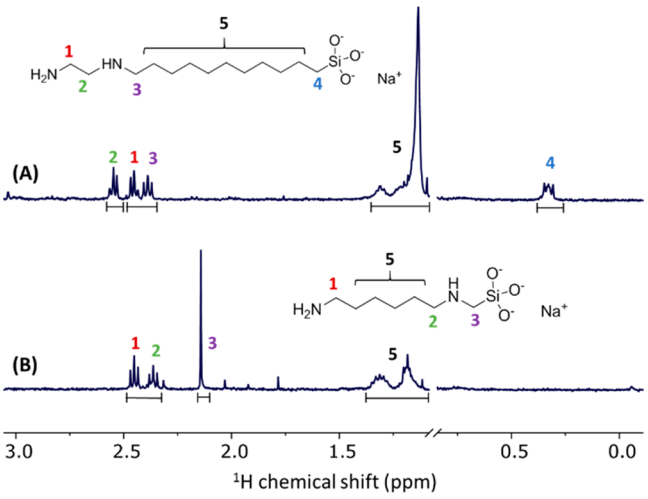

Figure 7. ^{1}H NMR spectra of 100 nm diameter (A) SiO_2@$C_{13}NH_2$ and (B) SiO_2@C_7NH_2 processed by the dissolution method. The EtOH resonance at 1.05 ppm was removed for clarity. The signals were assigned according to Chemdraw (CambridgeSoft).

these cases the quantification is complicated by the more complex ^{1}H NMR spectra. Only baseline-resolved signals that can be confidently assigned were selected as diagnostic signals for integration and quantification (see the Supporting Information).

In the case of both of the long-chain amines, only ≈40 μmol/g functional groups were attached, which represents ≈20% of the estimated monolayer coverage and is lower than the coverage obtained using APTES with the same reaction conditions (Table 2). This may be caused by the lower

Table 2. Functional-Group Quantification for Silica NPs from Different Sources and with Different Amine Structures As Measured by the Dissolution Protocol and NMR

silica NPs	qNMR (μmol/g)
NRC, SiO_2@C_7NH_2 100 nm[a]	43.5
NRC, SiO_2@$C_{13}NH_2$ 100 nm[a]	40.0
AQM, SiO_2@C_3NH_2 37 nm[b]	1230 ± 40
Nano and Amorphous Materials, SiO_2@C_3NH_2 20 nm[b]	19.5 ± 0.5
Superior Silica, SiO_2@C_3NH_2 200 nm[b]	270 ± 20

[a]One replicate, standard NMR. [b]Average of three replicates, qNMR.

reactivity of the silane precursors because the hydrolysis and condensation rates of different alkoxysilanes vary significantly with the substituent or steric factors associated with the longer alkyl chains.[13,15,16]

Finally, for comparison, the amine contents in silica NPs from three additional commercial suppliers were also measured (Table 2). Both the AQM and Superior Silica samples had amine contents substantially in excess of the values estimated for monolayer coverage for monodisperse NPs. By contrast, the Nano and Amorphous Materials sample had very low amine coverage, considering the high surface area for 20 nm NPs.

Hydrolytic Stability of Amine Functional Groups. 3-Aminopropyl-modified silicon oxide surfaces are known to be susceptible to hydrolysis, especially at elevated temperatures. Solution qNMR was employed to evaluate the degree of hydrolysis of amine-modified silica NPs. To achieve a measurable hydrolysis yield in a short time period, NPs of various sizes and with varying surface-group densities were heated at 56 °C for 30 min in D_2O and sonicated for 40 min in two intervals. These conditions were chosen as a typical procedure for elimination of endotoxins prior to cell-toxicity studies. After heating the samples, the amine contents on the NPs and in the solution were both determined. The sum of the two values (Table S4) was in reasonable agreement with the amine content obtained for the NPs prior to hydrolysis, confirming that most amines are accounted for. For the various amines tested, the functional-group loss varied from undetectable (based on amine detected in the supernatant, Table S4) to 60% (based on recovered NPs, Table 3). This trend appears to

Table 3. Loss of Amines from Silica NPs[a] after Dispersion by Sonication and Heating at 56 °C for 30 min[b]

	initial amine content (μmol/g)	final amine content (μmol/g)	percent loss
100 nm B3 SiO_2@C_3NH_2	190 ± 5	108	46%
100 nm NRC 0.2 SiO_2@C_3NH_2	35.5 ± 0.5	35	ND[c]
100 nm NRC SiO_2@C_7NH_2	44	38	12%
100 nm NRC SiO_2@$C_{11}NH_2$	40	42	ND
37 nm AQM027 SiO_2@C_3NH_2	1210 ± 60	520	60%
50 nm B2 SiO_2@C_3NH_2	215 ± 2	180	17%
50 nm B3 SiO_2@C_3NH_2	68 ± 2	64.6 ± 0.8	5%

[a]Measured from the ratio of recovered amine content to initial amine content. [b]See Table S4. [c]Amine not detected in supernatant.

be related to the surface density of the functional groups rather than the actual molar content or NP surface area. For example, the 100 nm (B3) and 37 nm AQM NPs had initial amine contents that were similar to or larger than the estimated monolayer coverage, and both lost approximately half the amine in 30 min at 56 °C. The 50 nm sized NPs with relatively high amine contents (215 μmol/g) lost ≈17% of the functional moieties, whereas much less amine loss occurred for 50 nm NPs with lower initial amine contents (68.0 μmol/g). Both samples with long-chain amines showed little or no amine loss under the same conditions. These samples had relatively low amine coverage, and additional experiments would be required to determine if the long-chain amines are also stable when present at higher coverage. The results are consistent with literature reports on planar silicon surfaces.[13,15,16]

The loss of functional groups will presumably be less rapid under ambient conditions, a more relevant situation for NP storage, or experiments in an aqueous environment. Therefore, we studied the kinetics of 3-aminopropyl siloxane group loss for the commercial sample with the highest surface functionalization (1230 μmol/g). The sample was equilibrated in water at room temperature for 90 h with periodic sampling

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of the supernatant for amine analysis. The results shown in Figure 8 indicate that 114 μmol of amine per gram of NPs is

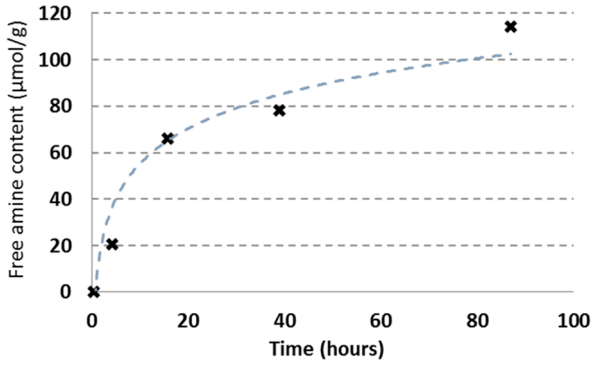

Figure 8. Kinetics of amine cleavage from 37 nm SiO_2@C_3NH_2 NPs (1.5 mg/mL, amine content of 1230 μmol/g) in D_2O at room temperature.

released, which represents almost 10% loss. These results demonstrate that exposure of amine-modified NPs to water should be limited. Otherwise, control experiments to determine amine loss should be carried out.

CONCLUSION

We have developed an optimized method for dissolving silica NPs for subsequent ^{1}H NMR quantification of the released amines. The detailed assessment of the effects of a range of variables on the dissolution of silica and the suitability of the samples for qNMR provide a framework for development of optimized protocols for other functional groups or for silicas that may require different conditions for dissolution. The method repeatability and its applicability to silica NPs with sizes ranging from 20 to 120 nm were assessed using commercial silicas with 3-amionopropyl functional groups from a single supplier. Analysis of multiple batches of commercial NPs indicated that the amine loading could vary by as much as a factor of 3 for different batches. These results clearly illustrate the need for methods to reliably and rapidly assess functional-group loading, especially for applications where the extent of surface coverage is expected to modulate the nanomaterial properties. The total amine content by qNMR was typically 20−30% lower than that measured by the colorimetric ninhydrin assay, which measures only reagent-accessible amines. Note that larger variations between accessible and total functional-group contents have been observed in other studies.[9,10] The qNMR method has the advantage of providing structural information on the released functional groups, in contrast to elemental analysis or inductively coupled plasma mass spectrometry, which provide elemental contents.

The dissolution−qNMR method was demonstrated to be compatible with longer-chain amines and with aminated silicas provided by a number of commercial suppliers, some of which were synthesized using modified procedures. This work clearly demonstrates that aminated silica nanoparticles from different suppliers differ dramatically in the surface coverage, with some materials having substantially more than the estimated monolayer coverage, indicating either significant porosity or multilayers of amine. By contrast, other silicas had ∼2% of the estimated monolayer coverage, which will have significant consequences for material properties. For example, low-amine-content silica will retain a net negative charge, whereas higher amine loading will result in a positive surface charge, potentially changing the localization of the material and its interaction with its environment. Some of the variability in amine content may reflect the inherent difficulties with using APTES for surface functionalization, because it is known to produce heterogeneous multilayers. Finally, the stability of aminated silicas was examined for several samples. Silicas functionalized with 3-aminopropyl groups undergo hydrolysis in water at room temperature, and there is significant amine loss at elevated temperatures. Examination of silicas with variable loading levels indicates that amines are more stable when present at lower densities on the silica surface.

EXPERIMENTAL SECTION

Chemicals. Deuterium oxide (99.9%), sodium deuteroxide (10 M in D_2O), 3-aminopropyltriethoxyilane (99%, APTES), TraceCERT maleic acid, and TraceCERT potassium phthalate monobasic for quantitative NMR were purchased from Sigma-Aldrich. Multiple batches of 20, 50, 80, 100, and 120 nm amine-modified SiO_2 NPs were purchased from Nano-Composix as ethanol suspensions (≈10 mg/mL), dry powder 37 nm SiO_2@C_3NH_2 was purchased from Applied Quantum Materials, dry powder 200 nm SiO_2@C_3NH_2 was from Superior Silica, and dry powder 20 nm SiO_2@C_3NH_2 was purchased from Nanostructured & Amorphous Materials. The mass fractions of silica in the NP suspensions were determined gravimetrically by drying at 125 °C to constant mass.

Instrumentation. All experiments were performed at 20 °C (±1 °C) with a Bruker Avance III 400 MHz spectrometer equipped with a 5 mm BBFO probe. A Ther-Mix heated mixer (Vitl Life Science Solutions) and an ultrasonic bath (Fisher Scientific) were used for sample preparation.

Standard ^{1}H NMR Experiments. Samples were placed in 5 mm borosilicate tubes (500 MHz) and inserted in the spectrometer. Shim values were loaded, and the sample was locked to D_2O, shimmed, and tuned. The measurement was conducted with the following parameters: 32 transients, 2 dummy transients, 30° pulse, 13 μs pulse length, 6 s relaxation delay, 20 ppm sweep width, 6 ppm O1. The acquired FIT spectrum was processed by Fourier transformation and phase- and baseline-corrected manually by fifth-order polynomial fit. This procedure was used for all spectra shown, with the exception of that in Figure 1; both standard and qNMR (see below) were used to quantify amines, and the figures and tables indicate the methods used.

^{1}H qNMR Experiments. For each analyte sample, the 90° pulse width was calibrated by determining the null signal generated by a 360° pulse width divided by 4 (in μs). The spin−lattice-relaxation time (T_1) was measured using an inversion-recovery experiment for each resonance of the analyte and the calibrant (maleic acid or potassium phthalate monobasic), and a delay of at least 7× the longest relaxation time for the components in the mixture was used. The maximum value for the receiver gain (RG) was attained. The ^{1}H NMR spectrum was then recorded using the 90° pulse program with the following parameters: 2 dummy transients, 32 transients, and 8 ppm spectral width with 3 ppm transmitter offset (for samples with maleic acid) or 12 ppm spectral width with 4 ppm transmitter offset (for samples with potassium phthalate monobasic). The spectrometer temperature was maintained at 20 °C. The acquired FIT spectra were processed

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by Fourier transformation and phase- and baseline-corrected manually by fifth-order polynomial fit.

Sample Preparation for NMR Measurements for Silica Dispersions. Amine-modified silica NPs (20 nm, 10 mg) were isolated from the stock dispersion in ethanol (5 mg/mL) by centrifugation (30 min, 14 500 rpm). The pellet was vacuum-dried overnight to remove the residual EtOH, and the NPs were dispersed in a deuterated solvent (EtOD-d_6/D_2O, 0.65 mL) by sonication in an ultrasonic bath for 60 s. The sample was transferred to a dry NMR tube and measured by the ^1H qNMR method. After the spectrum was recorded, the D_2O sample was equilibrated at RT over 100 min and then heated to 70 °C for 30 min. The sample was then cooled to 20 °C (±1 °C) over approximately 20 min, and the measurement was repeated. Although calibrant was added to quantify the amine loss, the signal quality was inadequate for qNMR.

Sample Preparation for Optimization of Dissolution Conditions. All analyzed samples were received as ethanol dispersions (10 mg/mL), from which NPs (5−10 mg) were isolated by centrifugation at 14 800 rpm for 15 min in 1.5 mL safe-lock Eppendorf microcentrifuge tubes. The supernatants were carefully removed from the pellets, which were then dispersed in 0.5 mL of D_2O and immediately centrifuged to remove residual EtOH. The D_2O supernatant was discarded, and the samples were dispersed in NaOD solution in D_2O (0.2/0.4 M) using an ultrasonic bath. Alternatively, NPs were isolated by slowly evaporating EtOH in a stream of air to form a thin film of NPs inside the microcentrifuge tube that was further dried under vacuum (≈3 mbar) over 16 h. The sample was dissolved in either an ultrasonic bath (60 min) or a heated shaker (1200 rpm) at 45 °C for 3 h. Maleic acid (MA) in D_2O (20 μL, 10 mg/mL) was used as an internal standard, because its resonance at 5.87 ppm does not interfere with the analyte signals.

Sample Preparation for qNMR−Dissolution Determination. An appropriate amount of solution (to provide a maximum of 12.5 mg of NPs) was pipetted into a 1.5 mL microcentrifuge tube, and the ethanol was evaporated in a gentle stream of air. The tube was dried under vacuum (≈3 mbar) for 16 h to remove residual solvent. For the dry silica NPs, the powders were weighed into 1.5 mL microcentrifuge tubes (maximum of 12.5 mg).

NaOD solution (0.650 mL, 0.4 M in D_2O) was added to the NPs, the tube was closed, and the solids were dispersed using an ultrasonic bath. The sample was placed into a heated mixer and shaken at 1200 rpm for 3 h at 45 °C. Heating the sample with vigorous shaking was necessary for complete dissolution of the NPs; otherwise, the aggregated NPs could precipitate from the solution, resulting in incomplete hydrolysis. The sample was then removed from the mixer and cooled to room temperature.

Maleic acid or potassium phthalate monobasic was used as internal calibrant in D_2O solutions (≈ 80 mM, prepared gravimetrically). Approximately 20 μL was added to the sample, and the exact mass gain was recorded. The tube was closed and vortexed for 5 s. The concentration of the calibrant was within the range of 0.2−5× the analyte concentration. The qNMR measurements were carried out within 24 h of sample preparation. Amine contents were determined by comparing the integrals of calibrant and the average of integrals of the selected diagnostic resonances. See the Supporting Information for guidelines on the selection of diagnostic resonances.

Ninhydrin Assay. A 500 μL aliquot of silica NP dispersion (containing ∼5 mg of NPs) was pipetted into a microcentrifuge tube and centrifuged at 14 000 rpm for 20 min. Ethanol (200 μL) was removed and replaced with 200 μL of water, and the sample was sonicated in a bath sonicator to redisperse the NPs. The sample was transferred to a glass test tube and 100 μL of KCN and 75 μL of phenol were added sequentially (from a Kaiser Kit, Anaspec). The tube was vortexed, and 75 μL of ninhydrin reagent was added. The tube was stoppered with a glass marble and placed in a preheated water bath at 97 °C for 10 min. The tube was removed, cooled in ice water, and poured into a 5 mL volumetric flask. The tube was rinsed with 60% EtOH, which was added to the volumetric flask to give a final volume of 5 mL. A 200 μL aliquot was placed in a centrifuge tube, diluted to a final volume of 1 mL in 60% EtOH, and centrifuged at 14 000 rpm for 20 min. The supernatant was then analyzed by UV−vis in a 1 cm path-length cell with dilutions as needed to ensure that the optical density at 570 nm was below 0.8. The assay was calibrated using the same procedure for a minimum of five concentrations of octyl amine.

Surface Functionalization of Bare SiO_2. Dry bare silica NPs (≈100 nm, NanoComposix) were dispersed in D_2O at a concentration of 10 mg/mL by 60 min bath ultrasonication in a glass vial. The sample was allowed to cool to room temperature and then was divided into four replicates. The appropriate amount of APTES (10%, v/v, APTES in EtOH, corresponding to 1.1, 0.5, 0.2, or 0.1× the estimated monolayer coverage of 180 μmol/g for 100 nm NPs) was added to the sample with stirring (1000 rpm). A blank sample without NPs was prepared as well. After the sample was stirred at room temperature for 1 h, the temperature was increased to 80 °C for 1 h. The solution was cooled, and the NPs were collected by centrifugation (15 min, 14 500 rpm) and washed three times by resuspending in D_2O. Purified NPs were concentrated by centrifugation to give a pellet that was dried in vacuo for 24 h prior to the dissolution−NMR analysis. Several analytical-scale experiments in D_2O for 1.1× APTES were carried out using the same procedure followed by NMR analysis of both isolated NPs and the supernatant to ensure that all amines were accounted for.

Stability of Aminated Silica. For experiments at elevated temperature, solid NPs (10 mg) were dispersed in D_2O (0.650 mL) by sonication in an ultrasonic bath for 20 min. The samples were placed in a heated shaker, shaken at 600 rpm for 30 min at 56 °C, and then frozen. Samples were thawed and then sonicated for 20 min, and NPs were separated from the solution by ultracentrifugation at 14 500 rpm for 10 min. The supernatant (0.6 mL) was carefully separated from the pellet, and concentrated NaOD was added to give a final concentration of 0.4 M to facilitate hydrolysis of potentially copolymerized siloxanes. The NP pellet was dispersed in NaOD (0.4 M, 0.6 mL) and both samples were hydrolyzed by a standard procedure in a heated mixer (1200 rpm for 3 h at 45 °C). After the samples were cooled, maleic acid calibrant solution was added, and standard ^1H NMR measurements were carried out within 24 h.

The stability of AQM 37 nm SiO_2@C_3NH_2 was evaluated at room temperature. The NPs were dispersed at 1.54 mg/mL in D_2O by probe sonication (180 J/mL) while being cooled in a water bath. The dispersion was stirred at RT, and 650 μL aliquots were collected at various time intervals. NPs were separated by centrifugation at 14 800 rpm for 10 min, the

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supernatant was carefully transferred into a separate microcentrifuge tube, concentrated NaOD was added to give a final concentration of 0.4 M, and MA calibrant solution was added. The sample was measured by qNMR within 24 h.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.8b02803.

Characterization of silica nanoparticles, NMR of silica nanoparticles, dissolution of silica NPs measured by UV−vis, optimization of silica mass and base concentration for dissolution of silica NPs and NMR analysis, characterization of APTES-functionalized silica NPs by NMR, characterization of in-house-functionalized silica NPs, selection of diagnostic signals for NMR analysis, and hydrolytic stability of functionalized silica NPs (PDF)

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Notes

The authors declare no competing financial interest.

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