Supplementary Information

Chain length of bioinspired polyamines affects size and condensation of monodisperse silica particles

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Supplementary Methods (Supplementary Note 1)

Materials: Tetraethyl orthosilicate (TEOS; ≥99%), Dietylenetriamine (C2N3; 99%), Triethylenetetramine (C2N4; ≥97%), Sodium hydroxide pellets (≥98%), Triethylamine (≥99.5%), L-lysine (≥98%) were purchased from Sigma Aldrich. Ethylamine (70% solution in water), Tetraethylenepentamine (C2N5) and Pentaethylenehexamine (C2N6) were purchased from TCI, Netherlands. All chemicals were used as received. Deionized water (Milli-Q®; Merck) was used for synthesis.

Silica particle synthesis with varying TEOS amounts: Silica particles were synthesized by modifying the method1 developed by Yokoi et al. 22 mM polyamine solutions were heated to 60°C and equilibrated for 30 minutes. Different amounts of TEOS were added to the reaction mixture and the reactions were continued until the TEOS was completely hydrolysed. The reaction time1,2 was chosen to ensure complete hydrolysis of TEOS and condensation of silicic acid to particles. The particle dispersions centrifuged...
(12000 rpm, 10 minutes), and the isolated pellets were freeze-dried. The molar ratio of the reactions was $z$ TEOS: 1C2Nx: 2523 H2O where $z = 0.90, 1.1, 3.4, 9.1, 11.4$ and 15; $x = 3, 5, \text{and } 6$.

**Characterization**: The size of the silica particles was determined from SEM images of the particles. Briefly, 10 µL of the particle dispersion was deposited on a silicon wafer (preheated to 60°C) and dried at 60°C for 1 hour. The resultant film was then sputter-coated with platinum (Quorum Q150T plus turbo pumped sputter coater; UK) and imaged using SEM Quanta 3D FEG (TFS, USA), at an acceleration voltage of 20 kV. Average Silica particle diameter was determined using an in-house MATLAB script with 100 particles measured for each of the reaction conditions. For EDS elemental mapping, 50 µl of the silica particle suspension was dropped onto a standard 200 mesh copper TEM grid covered by a 10 nm continuous carbon film. TEM grids were placed in an in-house made sample holder suitable for an SEM stage. SEM-EDS mapping was performed using SEM Quanta 3D FEG (Thermo Fisher Scientific, USA), at an acceleration voltage of 10 kV. Zeta potential measurements were carried out on 1.5 mg/mL silica particle dispersions using Anton-Paar Litesizer 500 (Anton-Par, Austria). TGA measurements (TA instruments, Germany) were carried out on freeze-dried particles. The 4 to 6 mg of freeze-dried silica particle powders were heated in platinum pans from 30°C to 1000 °C at 20°C/ min in air.

**Solid-state NMR**: Measurements were performed on an Oxford magnet of a static magnetic field at 9.4 T (operating frequency for $^1$H and $^{29}$Si at 400.1 MHz and 79.5 MHz, respectively) equipped with a Bruker NEO spectrometer. All samples were packed into either a 6 mm (o. d.) PENCIL rotor with two Teflon restrictors or a 1.6 mm (o. d.) PENCIL rotor and measured with Varian 6 mm or 1.6 mm HXY probe heads. Nitrogen gas was used to spin the rotor, and the temperature was regulated at 15°C during measurement to avoid further silica condensation. $^{29}$Si and $^1$H $T_1$ relaxation time constants were measured using saturation-recovery experiment. For 1D $^{29}$Si MAS NMR spectra, magic-angle spinning (MAS) rate was set to 5 kHz, and the recycle delays, typically between 1200 and 2000 seconds, were set to 5 times of $^{29}$Si $T_1$. High power proton decoupling was applied using SPINAL64 scheme with a $^1$H r.f. at ca. 62.5 kHz during signal acquisition. Some number between 110 and 150 of transients were averaged to afford spectra with a decent signal-to-noise ratio. For spectra processing 50 Hz of apodization was applied. The $^{29}$Si chemical shift is externally referenced to tetramethylsilane (TMS) at 0 ppm. SSNake software was used to afford spectra fitting result. For 1D $^1$H MAS NMR spectrum, MAS rate was set to 30 kHz, and the recycle delay was set to 5 times of $^1$H $T_1$. For 2D $^{29}$Si-$^1$H heteronuclear correlation (HETCOR) experiments, a ramped cross polariton (CP) scheme was used with a ramp profile between 90% and 100 % on $^1$H. MAS rate was set to 30 kHz, and the recycle delay was set to 1.3 times of $^1$H $T_1$. CP contact times were set to 0.1 ms or 3 ms , and
high power proton decoupling was applied using SPINAL64 scheme with $^1$H r.f. power at ca. 100 kHz during signal acquisition. 8192 and 512 transients were averaged per $t_1$ increment for short and long CP contact times, respectively. STATES-TPPI method was used, and 16 $t_1$ increments were collected. 200 Hz and 10 Hz of apodization were applied on $^{29}$Si and $^1$H dimension, respectively.
Supplementary Note 2: Cryo-TEM images of particles produced at pH 11 using simple bases

Figure S2: Cryo-TEM images of silica particles produced in presence of simple bases at initial pH of 11; initial pH measured at room temperature. (a) NaOH; (b) Ethylamine (44 mM); (c) Triethylamine (44 mM); (d) L-Lysine (22 mM); (e) L-Lysine (44 mM) [Scale bar 50 nm].
Supplementary Note 3: Additional SEM images of silica particles synthesized at different polyamine concentrations

Figure S3: SEM images of silica particles synthesized at different polyamine concentrations (Scale bars: 1 µm). The reactions were carried out overnight.
**Table S3:** Size of silica particles produced in presence of different polyamines. Average Particle diameters (± Standard deviation) were measured using in-house MATLAB script, 100 particles were measured for each sample.

| Polyamine                      | 22 mM (Polyamine conc.) | 33 mM (Polyamine conc.) | 44 mM (Polyamine conc.) |
|--------------------------------|-------------------------|-------------------------|-------------------------|
| **Diethylenetriamine** (C2N3) | 137±13 nm               | 100±15 nm               | 83±15 nm                |
| **Triethylenetetramine** (C2N4)| 333±17 nm               | 255±13 nm               | 225±17 nm               |
| **Tetraethylenepentamine** (C2N5)| 479±16 nm               | 365±23 nm               | 280±17 nm               |
| **Pentaethylenehexamine** (C2N6)| 617±20 nm               | 512±17 nm               | 420±27 nm               |
Supplementary Note 4: Effect of TEOS concentration on silica particle size at constant polyamine concentration

Table S4a: Average particle diameters of silica particles produced at varying TEOS_Polyamine mole ratios. Average Particle diameters (± standard deviation) were measured using in-house MATLAB script, 100 particles were measured for each sample.

| Sample id      | C2N3 (nm) | C2N5 (nm) | C2N6 (nm) |
|----------------|-----------|-----------|-----------|
| 0.9T_1C2Nx     | 70±7      | 235±35    | 368±56    |
| 1.1T_1C2Nx     | 68±6      | 248±25    | 358±41    |
| 3.4T_1C2Nx     | 74±8      | 316±17    | 381±43    |
| 9.1T_1C2Nx     | 102±11    | 418±29    | 511±35    |
| 11.4T_1C2Nx    | 108±12    | 429±46    | 516±37    |
| 15T_1C2Nx (Final concentration) | 137±13 | 479±16 | 617±20 |

Predicted particle diameter: Predicted particle diameter was determined using the following equation (Supp. Eq. 1):

\[
\text{Predicted diameter} = \frac{\text{Final diameter} \times \text{Silicic acid concentraton}}{\text{Final Silicic acid concentration}}
\]

Final Silicic acid concentration = 330 mM

Silicic acid concentration at various zTEOS_1C2Nx mole ratios is given below in Table S4b
Table S4b: Silicic acid concentrations at various mole ratios.

| Sample id         | Silicic acid concentration |
|-------------------|-----------------------------|
| 0.9T_1C2Nx        | 20 mM                       |
| 1.1T_1C2Nx        | 25 mM                       |
| 3.4T_1C2Nx        | 75 mM                       |
| 9.1T_1C2Nx        | 200 mM                      |
| 11.4T_1C2Nx       | 250 mM                      |
| 15T_1C2Nx (Final concentration) | 330 mM          |
Supplementary Note 5: SEM images of silica particles produced at low concentration

The SEM samples were produced by depositing 10 µL sample on silicon wafer preheated to 60°C. These sample preparation steps were adopted to avoid artefacts that might result from cooling the reaction.

**Figure S5:** a) c) and e) SEM images of silica oligomer aggregates at 0.50TEOS_1C2N6 mole ratio, and particles at 0.57 and 0.70TEOS_1C2N6 mole ratios respectively; b) and d) particles were not observed at 0.50 and 0.57TEOS_1C2N3 mole ratio; f) SEM images of particles observed at 0.70TEOS_1C2N3.
Supplementary Note 6: Cryo-TEM silica particles produced at presence of C2N3(Diethylene triamine) at 0.70TEOS_1C2N3– Time course measurement

**Figure S6**: Average particle diameter of silica particles produced in presence of 22 mM C2N3 at 0.70TEOS_1C2N3 mole ratio; Average particle diameter determined from Cryo-TEM images by measuring 100 particles (error bars represent standard deviation).
**Supplementary Note 7: Energy Dispersive X-Ray spectroscopy (EDS)**

**Figure S7a**: SEM-EDS map of silica particle produced in presence of C2N6 at 15T_1C2N6 mole ratio showing the presence of carbon and nitrogen along with silicon and oxygen indicating co-localization of polyamines with silica.

**Figure S7b**: SEM-EDS map of FIB milled silica particle produced in presence of C2N6 at 15T_1C2N6 mole ratio showing the presence of carbon and nitrogen along with silicon and oxygen indicating internal co-localization of polyamines with silica.
Supplementary Note 8: Infra-Red spectroscopy

**Figure S8**: FTIR spectrum of C2N6 (Orange) and freeze dried 617 ± 20 nm silica particles grown in presence of 22 mm C2N6 (black; 15T_1C2N6). FTIR spectrum of silica produced in absence of polyamines (blue) provided for reference.
Supplementary Note 9: Thermo Gravimetric Analysis

Figure S9: Normalized TGA curves of silica particles grown at 15TEOS_1C2Nx mole ratio obtained by heating the samples in air. Samples were heated from RT to 1000°C at 20°C/min. Sample weight-loss was normalized from 160°C to account for adsorbed water.
**Supplementary Note 10: 1D NMR Spectra**

**Figure S10a:** $^1$H NMR spectrum of silica particles produced at 15T\_1C2N6 mole ratio.

**Figure S10b:** Silicon environments (in red) identified from $^{29}$Si MAS spectra of freeze-dried silica particles.
**Table S10**: $^{29}$Si Chemical shifts and relative amounts obtained from quantitative $^{29}$Si MAS NMR

| Sample     | Q4 ppm (%) | Q3 ppm (%) | Q2 ppm (%) |
|------------|------------|------------|------------|
| 0.9T_1C2N6 | -110 ppm (47.3%) | -100 ppm (41.4%) | -92.5 ppm (11.3%) |
| 3.4T_1C2N6 | -110 ppm (49.5%) | -100 ppm (42.4%) | -90.6 ppm (8.1%) |
| 9.1T_1C2N6 | -110 ppm (52.7%) | -100 ppm (40.6%) | -90.6 ppm (6.8%) |
| 15T_1C2N6  | -110 ppm (55.9%) | -100 ppm (38.1%) | -91.2 ppm (6.0%) |
| 15T_1C2N3  | -111 ppm (63.2%) | -100 ppm (32.4%) | -90.9 ppm (4.4%) |

**Figure S10c**: $^{29}$Si NMR of silica particles produced at 15T_1C2N3 mole ratio.
Supplementary Note 11: 2D $^{29}\text{Si}^{-1}\text{H}$ HETCOR

Figure S11: 2D $^{29}\text{Si}^{-1}\text{H}$ HETCOR NMR with CP contact time of 0.1 ms of silica particles produced at 15T_1C2N6 mole ratio.
**Supplementary Note 12: pKa values of amines**

**Table S12**: pKa values of the polyamines.

| Polyamine             | pKa 1 | pKa 2 | pKa 3 | pKa 4 | pKa 5 | Ref |
|-----------------------|-------|-------|-------|-------|-------|-----|
| Ethylamine            | 10.8  |       |       |       |       | 5   |
| Triethylamine         | 10.8  |       |       |       |       | 5   |
| Ethylene diamine      | 10.1  | 7.0   |       |       |       | 5   |
| Diethylene triamine   | 9.9   | 9.1   | 4.3   |       |       | 6   |
| Triethylene tetramine | 9.9   | 9.2   | 6.7   | 3.3   |       | 6   |
| Tetraethylene pentamine| 9.9  | 9.1   | 7.9   | 4.3   | 2.7   | 6   |
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