Electronic Supplementary Material (ESI) for ChemComm.
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Electronic Supplementary Information (ESI)

Emulsion-templated silica nanocapsules formed using bio-inspired silicification

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Experimental section

Synthesis of silica nanocapsules: To synthesize silica nanocapsules, lyophilized SurSi peptide (Peptide 2.0 Inc., VA) was dissolved in sodium 4-(2-hydroxyethyl)-1-piperazine ethanesulfonate (HEPES) buffer (25 mM, pH 7) in the presence of $ZnCl_2$ (800 μM) to give a final peptide concentration of 400 μM. Then Miglyol® 812 (20 μL) was added into 1 mL of the solution. The mixture was sonicated at 10 W (Branson Sonifier 450 ultrasonicator) for four 30 s burst and interspersed in an ice bath for 60 s. Nanoemulsion (1 mL) was dialyzed against HEPES buffer (25 mM, 500 mL, pH 7.5) at 4°C for 20 h. An aliquot of the nanoemulsion (400 μL) was transferred into a 4-mL glass vial for biosilicification reaction with tetraethoxysilane (TEOS) under magnetic stirring for up to 50 h at room temperature. To investigate the effects of reaction time and silica precursor concentration, TEOS concentration was varied and samples were taken at various times for characterization. A control vial demonstrated stability of the nanoemulsion for the reaction period.

Encapsulation efficiency: Encapsulation efficiency of fipronil (%*EE*) was characterized by the percentage between mass of fipronil in silica nanocapsules after the final TEOS removal step and initial mass of fipronil dissolved in the oil phase. To calculate %*EE*, silica nanocapsules containing fipronil were prepared as previously described. After TEOS removal step, the suspensions of silica nanocapsules were freeze-dried at 0.08 mbar and –55 °C for 20 h. The freeze-dried samples were mixed with water, finely dispersed by sonication at 20 W

(Branson Sonifier 450 ultrasonicator) for 60 s, and subsequently added into acetonitrile with final acetonitrile concentration of 60 v/v%, as fipronil is soluble in acetonitrile (60 v/v%) as high as 71.5 mg mL^{-1}, under vigorous stirring at room temperature for up to 72 h. The suspensions were centrifuged at an interval 24 h and the supernatants were collected, then fresh acetonitrile (60 v/v%) was added to the pellets. The releases of fipronil in the acetonitrile solutions were repeated until no residual fipronil in supernatants was detected. The fipronil concentrations in the supernatants were measured by RP-HPLC as described in the following section. Then, the encapsulation efficiencies of fipronil were calculated by using Eq. S1, where C_f is the fipronil concentration in release medium (mg mL^{-1}), V_f is the volume of release medium (mL), C_i is the initial fipronil concentration in the Miglyol® 812 oil (10 mg mL^{-1}), V_i is the volume of the oil phase (mL) and n is the number of repeated-release of fipronil.

$$\text{Encapsulation Efficiency: } \%EE = \frac{\sum_{j=1}^{n} (C_f V_f)_j}{C_i V_i} \times 100\% \qquad \text{(Eq. S1)}$$

Sustained release study: To encapsulate fipronil, fipronil was first dissolved in Miglyol® 812 (10 mg mL^{-1}) used to form a nanoemulsion. Fipronil-loaded silica nanocapsules were then prepared by a reaction of dialyzed fipronil-loaded nanoemulsions with TEOS at concentration of 16 μmol, 32 μmol, or 96 μmol in 25 mM HEPES buffer at pH 7.5 for 30 h. The 10 mg mL^{-1} fipronil-loaded nanocapsules with different silica shell thicknesses were then dialyzed in water at 4°C for 20 h to remove residual TEOS. Then, the release study was started by stirring the nanocapsules aqueous solution at room temperature. At different time intervals, aliquots of 200 μL were taken, replaced with water, and the aliquots were centrifuged to obtain clear supernatant. Fipronil in the supernatant was assayed by high-performance liquid chromatography (HPLC) using a Shimadzu system equipped with a reversed-phase C18 column (Jupiter, 5 μm, 300 Å, 150 × 4.6 mm). Phosphoric acid 0.1 v/v% (A) and 90 v/v% acetonitrile, 0.1 v/v% phosphoric acid (B) were used as the mobile phases. A linear gradient from 50 to 70% B in 20 min at a flow rate of 1 mL min^{-1} was used with a monitoring wavelength set at 220 nm. The concentrations of fipronil released were reported relative to the saturation limit of fipronil in water (2 μg mL^{-1}). To determine the concentration of

fipronil, a standard curve was obtained by dissolving weighted amounts of fipronil powder in 60 v/v% acetonitrile.

Characterization: Dynamic light scattering (DLS) was performed on Malvern Zetasizer Nano ZS at a scattering angle of 173° and a temperature of 25°C. Samples were diluted by a factor of 100 prior to the size and zeta potential measurements. Transmission electron microscopy (TEM) was carried out on a JEOL 1010 operated at 100 kV. Samples (2 µL) were deposited onto Formvar-coated copper grids, washed with water and left to air-dry prior to TEM examination, without the need for staining or cryopreservation. Energy dispersive X-ray spectroscopy (EDX) spectrum were obtained by using high-resolution (HR) TEM (JEOL 2100, 200 kV accelerating voltage) equipped with an EDX detector.

Supplementary figures

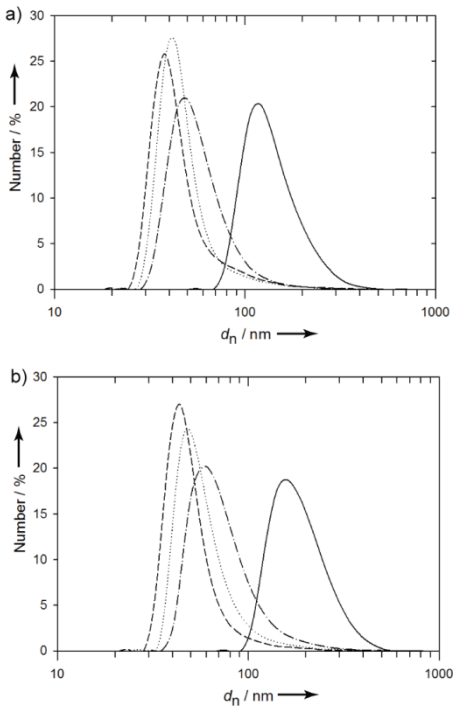

Fig. S1 (a–b) Size distribution of SurSi-stabilized nanoemulsions and nanocapsule formed at pH 7.5 (a) and pH 8 (b) as determined by dynamic light scattering (DLS), after emulsification at pH 7 (– – –), after 20 h of dialysis at pH 7.5 (a) or pH 8 (b) (·····), after 20 h of reaction with 32 µmol tetraethoxysilane (TEOS) at pH 7.5 (a) or pH 8 (b) (——), and control SurSi-stabilized nanoemulsion after 40 h of incubation at pH 7.5 (a) or pH 8 (b) (–·–·–·).

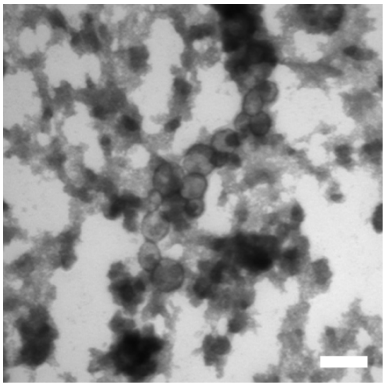

Fig. S2 Transmission electron microscopy (TEM) image taken after a 20-h reaction of the dialyzed AM1-stabilized nanoemulsions with 32 μmol tetraethoxysilane (TEOS) in 25 mM HEPES buffer pH 7.5 at room temperature. Scale bar is 200 nm.

The procedure for the synthesis of silica nanocapsules by templating AM1-stabilized nanoemulsions was described in the *Synthesis of silica nanocapsules* (**Experimental section**) except that AM1 peptide was used instead of SurSi peptide. Despite the formation of silica nanocapsules by templating AM1-stabilized nanoemulsions, it led mainly to the formation of bulk silica nanoparticles (Fig. S2) indicating that the dual functionality of emulsion stabilization and silica shell formation cannot be effectively achieved by using AM1 as its surface activity is sacrificed during biosilicification reactions resulting in formation of silica largely in bulk solutions (Fig. S2).

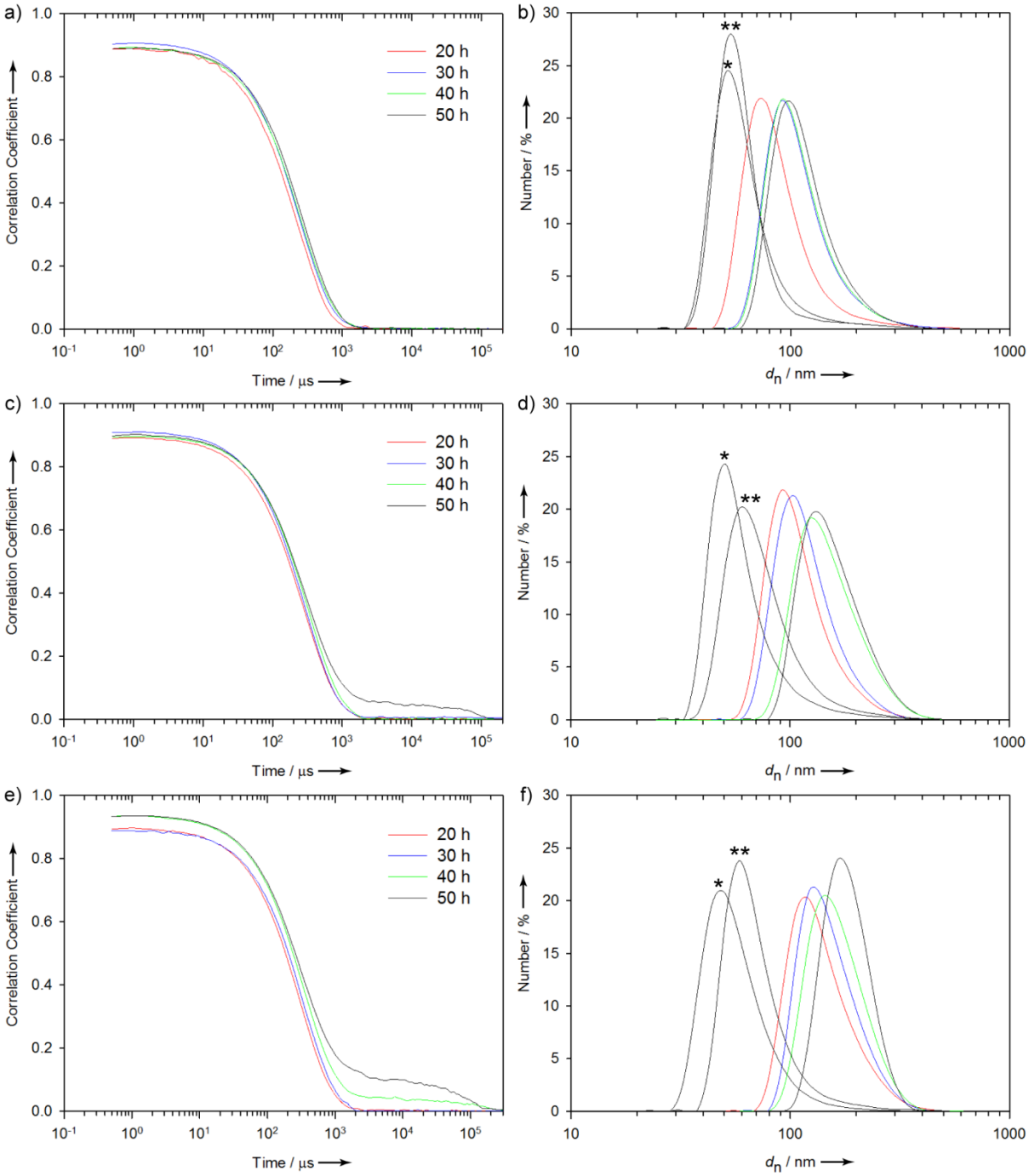

Fig. S3 Dynamic light scattering (DLS) results presenting the correlograms (left) and the associated number-weighted size distributions (right). (a–f) DLS data of the silica nanocapsules were taken after reaction of SurSi-stabilized nanoemulsions with tetraethoxysilane (TEOS) at concentrations of 8 µmol (a, b), 16 µmol (c, d) and 32 µmol (e, f) in 25 mM HEPES buffer pH 7.5 for up to 50 h at room temperature. Samples of SurSi-stabilized nanoemulsions before reaction with TEOS (*) and dialyzed SurSi-stabilized nanoemulsions incubated for 50 h (**) are included in the size distribution profiles (right) for comparison.

The correlograms show a typical single exponential decay of the scattered intensities over time due to the Brownian motion of the nanocapsules (Fig. S3a, c, e). However, the correlograms of the samples of silica nanocapsules obtained after reactions with 16 µmol TEOS for 50 h (Fig. S3c), and 32 µmol TEOS for 40 h and 50 h (Fig. S3e) show multiple peaks, i.e., substantial tail and non-flat baseline at the end of the decay, indicating the presence of aggregates. The decays of these samples show more extended slope suggesting their polydispersities. These are confirmed by the polydispersity index (PDI) values presented in Table S1. The number-weighted size distributions, which were calculated from the correlation functions by the zetasizer software, show only single peaks suggesting that the corresponding aggregates were present in trace amounts (Fig. S3b, d, f). Furthermore, the time at which the correlation function starts to decay over time gives an indication of the mean size of the nanocapsules in the suspensions. Increasing reaction times between SurSi-stabilized nanoemulsions and TEOS produces larger silica nanocapsules which move more slowly in the suspensions and thus the correlation functions persist for longer time prior to the decay (Fig. S3a, c, e). The number-weighted size distributions show more clearly the increase in size as the reaction times were increased (Fig. S3b, d, f).

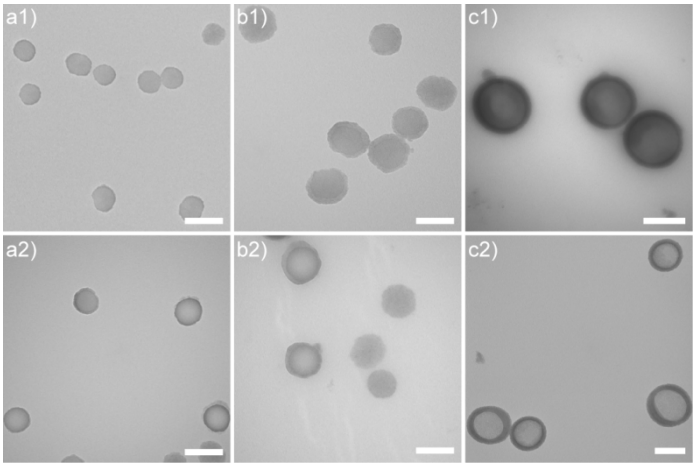

Fig. S4 (a–c) Transmission electron microscopy (TEM) images of the silica nanocapsules having shell thickness of 8 nm (a), 25 nm (b) and 44 nm (c) before (1) and after (2) fipronil-release study. Scale bars are 200 nm.

Supplementary table

Table S1 Polydispersity index of nanocapsules as a function of reaction time and tetratethoxysilane (TEOS) concentration.

Time / **mol_{TEOS}**	**0 h**	**20 h**	**30 h**	**40 h**	**50 h**
0 µmol	0.293	0.318	-	-	-
8 µmol	-	0.216	0.184	0.150	0.140
16 µmol	-	0.168	0.140	0.154	0.345
32 µmol	-	0.146	0.180	0.468	0.651