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Electronic Supplementary Information

Inorganic-Organic Coprecipitation: Spontaneous Formation of Enclosed and Porous Silica Compartments with Enriched Biopolymers

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Experimental Procedures

1.1 Materials

All chemical reagents were used without further purification. Tetraethyl orthosilicate (TEOS), tetramethoxysilane (TMOS), tetrapropyl orthosilicate (TPOS), NaCl (GR grade), D-(+)-glucose (GC grade, 99.5%), 4-aminoantipyrine (98%) were purchased from Aladdin. Calf thymus deoxyribonucleic acid (CT-DNA, BR grade, 98%), $CaCl_2$ (AR grade, 96%) were purchased from Shanghai Macklin Biochemical Co., Ltd. Bovine serum albumin (BSA, BR grade, 96%) was purchased from Beijing Solarbio Science & Technology Co., Ltd. Glucose oxidase (GOD, BR grade, 100-250 u/mg), horseradish peroxidase (POD, BR, 300 u/mg), N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine (98%) were purchased from Shanghai Yuanye Bio Technology Co., Ltd. NaOH (AR grade) was purchased from Guangzhou Jinhuada Chemical Reagent Co., Ltd. Isopropanol (IPA, AR grade), methanol (MeOH, GR grade), ethanol (EtOH, AR grade), $NH_3 \cdot H_2O$ (AR grade, 25-28% w/w), KOH (AR grade), $FeCl_3$ (AR grade), $CuCl_2$ (AR grade) were purchased from Sinopharm Chemical Reagent Co., Ltd. Deionized water (resistance > 18.2 $M\Omega/cm^{-1}$) was used in all reactions. Copper specimen grids (200 mesh) with formvar/carbon support film (referred to as TEM grids in the text) were purchased from Beijing Zhongjingkeyi Co, Ltd.

1.2 Characterization

TEM images were collected on a FEI Talos L120C TEM operated at 120 kV. HR-TEM, SAED, HAADF-TEM and EDS elemental mappings were performed on a JEOL JEM-2100F TEM operated at 200 kV. SEM images were collected from a FEI Quanta 250 FEG STEM operated at 20 kV. The CD spectra was obtained on a Rudolph Autopol IV-T Circular Dichroism spectropolarimeter and data were collected with scanning rate of 120 nm/min ranging from 190 to

360 nm with step of 1.0 nm. The element content was determined on a PerkinElmer Avio™ 200 ICP-OES and a Thermo Fisher iCAP RQ ICP-MS. Thermogravimetric data were obtained from a Mettler Toledo TGA/DSC 3+/1600 HT simultaneous thermal analyzer and carried out under a 50 mL/min flow of air with a temperature ramp of 10 °C/min from 30 °C to 900 °C. Enzymatic activity was measured on a Tecan Infinite M200 PRO microplate reader.

1.3 Synthesis of silica compartments

$CaCl_2$ (0.6 M, 5 μL), $NH_3{\cdot}H_2O$ (40 μL) and TEOS (2 μL) were added successively into an IPA/water mixture (V/V = 5:1, 1.4 mL) with stirring. The solution gradually changed from transparent to turbid white, indicating the nucleation and growth of silica. After stirring at room temperature for 12 h, the resulting products were diluted with water (200 μL diluted by 1.1 mL H_2O) and then purified via centrifugation at 10000 g for 7 min. In this method, isopropanol is used to reduce the solubility of poly(silicic acid) chains and promote their nucleation; the salt and base provide sufficient counter ions (Ca^{2+} and NH_4^+) for the formation of silica, as we reported previously.[1] The experiment can be scaled up.

1.4 Synthesis of silica compartments in various mixture reagents

The different mixture systems are as follows:

1) IPA (1.16 mL), H_2O (240 μL), $CaCl_2$ (0.6 M, 5 μL), $NH_3{\cdot}H_2O$ (40 μL), TEOS (2 μL), TMOS (1 μL), TPOS (1 μL), (Figure S8a and b)
2) IPA (1.16 mL), H_2O (240 μL), $CaCl_2$ (0.6 M, 5 μL), $NH_3{\cdot}H_2O$ (30 μL), NaOH (0.02 g/mL, 10 μL), KOH (0.02 g/mL, 10 μL), TEOS (2 μL), (Figure S8c and d)
3) IPA (1 mL), EtOH (320 μL), MeOH (200 μL), H_2O (240 μL), $CaCl_2$ (0.6 M, 5 μL), $NH_3{\cdot}H_2O$ (40 μL), TEOS (2 μL), (Figure S8e)
4) IPA (1.1 mL), EtOH (100 μL), MeOH (100 μL), H_2O (100 μL), $CaCl_2$ (0.6 M, 5 μL), $NH_3{\cdot}H_2O$ (40 μL), TEOS (2 μL), (Figure S8f)
5) IPA (1.16 mL), H_2O (240 μL), $CaCl_2$ (0.6 M, 5 μL), $FeCl_3$ (0.1 M, 3.5 μL), $CuCl_2$ (0.1 M, 7 μL), $NH_3{\cdot}H_2O$ (40 μL), TEOS (2 μL), (Figure S8g and h)
6) IPA (1.1 mL), EtOH (100 μL), MeOH (100 μL), H_2O (100 μL), $NH_3{\cdot}H_2O$ (30 μL), NaOH (0.02 g/mL, 10 μL), KOH (0.02 g/mL, 10 μL), $CaCl_2$ (0.6 M, 9 μL), $FeCl_3$ (0.1 M, 2.5 μL), $CuCl_2$ (0.1 M, 5 μL), TEOS (2 μL), TMOS (1 μL), TPOS (1 μL), (Figure S8i)

Each group of the mixture system was stirred at room temperature for 12 h. The resulting products were diluted with water and then centrifuged at 10000 g for 7 min to remove the supernatant for further characterization. The experiments all can be scaled up.

1.5 Synthesis of silica compartments with solvent evaporation

IPA (0.58 mL), H_2O (120 μL), $CaCl_2$ (0.6 M, 2.5 μL), $NH_3{\cdot}H_2O$ (20 μL) and TEOS (1 μL) were added successively into an 8 mL vial with stirring. The reaction was incubated for 14 h without bottle cap so that solvents can be evaporated during the synthesis process. The resulting sample was redispersed in 4 mL water. After 2 h, it was centrifuged at 10000 g for 7 min to remove the supernatant for further characterization (Figure S9).

1.6 Synthesis of silica compartments with enriched biopolymers

$NH_3{\cdot}H_2O$ (40 μL), $CaCl_2$ (0.6 M, 3.5 μL), were added successively into IPA (1.16 mL). As adding DNA (dissolved in H_2O, 0.5 mg/mL) or protein (dissolved in H_2O, 0.75 mg/mL), the solution immediately changed from transparent to turbidity, indicating DNA or protein nucleated out. Then, TEOS (2 μL) were added. After stirring at room temperature for 12 h, the resulting products were diluted with water and then centrifuged at 10000 g for 7 min to remove the supernatant for further characterization. The experiments all can be scaled up.

1.7 Measurement of element content

The content of Ca and Si in silica was determined by ICP-OES. The silica nanoparticles were dissolved in ammonia solution (2.24 M, 6 mL) and heated at 90 °C for at least 3 h. TEM characterization showed no silica nanoparticles after the etching, indicating the complete digestion of the sample. The solution was neutralized with HCl where the pH is 5-7, and then used for ICP-OES measurement. Two parallel experiments were carried out to determine the molar ratio of Ca/Si in silica. The results are shown as following table S1.

Based on a similar sample digestion procedure, the content of P from CT-DNA trapped in silica compartments was determined by ICP-MS. The only difference is that this solution is not neutralized with acid before the characterization.

Table S1 The ICP-OES results showing the molar ratio of Ca/Si in silica at different synthesis stages.

	Experiment 1	Experiment 2
The intermediates trapped at 20 min	0.918	0.894
As-prepared products after 12 h	0.298	0.291
Silica shells after etching	0.140	0.119

1.8 TGA of trapped protein in silica

GOD-trapped silica compartments (GOD@silica) were prepared via the above method. The corresponding total amount of GOD used in the synthesis is 1.03 mg. As prepared sample was transferred to a 4 mL glass vial whose

weight is known, dried at 80 °C for 2 days, and then weighed, giving a sample weight of 6 mg. Weigh 3.80 mg sample for TGA. The weight loss is 43.0978%, as shown in the Figure S17a.

The plain silica was prepared through the same synthesis and purification process as a control. As shown in Figure S17a, the weight loss of plain silica is 39.3515%.

The neat GOD was also characterized by TGA, showing a weight loss of 88.557% (Figure S18a). Considering the reagent purity of GOD we used is 95%, the actual weight loss of GOD is about 93%.

Using m_{GOD} and m_{silica} as the mass of GOD and silica in the sample, respectively. According to $m_{GOD} + m_{silica} = 3.8$ and $93\% \cdot m_{GOD} + 39.3515\% \cdot m_{silica} = 3.8 \times 43.0978\%$, the content of GOD can be calculated as 0.26 mg. The loading content is defined as the mass ratio of the trapped GOD to the GOD@silica sample, and the enrichment efficiency is defined as the ratio of the trapped GOD over the total amount of GOD used in the synthesis, as follows:

$$\text{loading content} = \frac{\text{the mass of trapped biopolymer in silica}}{\text{the mass of biopolymer@silica}}$$

$$\text{enrichment efficiency} = \frac{\text{the mass of trapped biopolymer in silica}}{\text{the mass of biopolymer used in synthesis}}$$

By further calculation, the loading content of GOD is 6.8% and the enrichment efficiency is 39.6%.

The same analysis method was used for determining the content of BSA, giving the loading content of 12.2% and the enrichment efficiency of 49.1%.

To define λ as the ratio of the concentration of biopolymers inside the silica cavity over the initial concentration in synthesis solution, it can be calculated as follows:

$$\lambda = \frac{\text{the concentration of biopolymers inside the silica cavity}}{\text{initial concentration of biopolymer in synthesis solution}}$$

$$= \frac{\text{the mass of trapped biopolymer / the volume of silica compartments}}{\text{the mass of biopolymer used in synthesis / the volume of synthesis solution}}$$

$$= \text{enrichment efficiency} \times \frac{\text{the volume of synthesis solution}}{\text{the volume of silica compartments}}$$

1.9 Enzymatic activity of GOD

The assay mixture contained glucose (0.125 M in H_2O, 8 mL), POD solution (25 unit/mL in H_2O, 2 mL), 4-aminoantipyrine (0.5% in H_2O, 1.2 mL), and N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine (20 mM in H_2O, 0.8 mL). To assay the enzymatic activity of GOD, 10 μL of the test sample and 190 μL of assay solution were mixed, and then reacted to produce quinoneimine dye. The color change within the initial six minutes was monitored at 555 nm by UV-Vis extinction on a Tecan microplate reader. Each sample is tested repeatedly at least five times. One unit of enzymatic

activity of GOD was defined as the amount of enzyme that oxidize 1 μmol glucose (equivalent to release half a micromole of quinoneimine dye) per minute. The enzymatic activity of GOD@silica dispersion and free GOD solution from 1 to 3 minutes were compared. Therein, content of GOD in both mixtures was the same.

Supplementary Figures

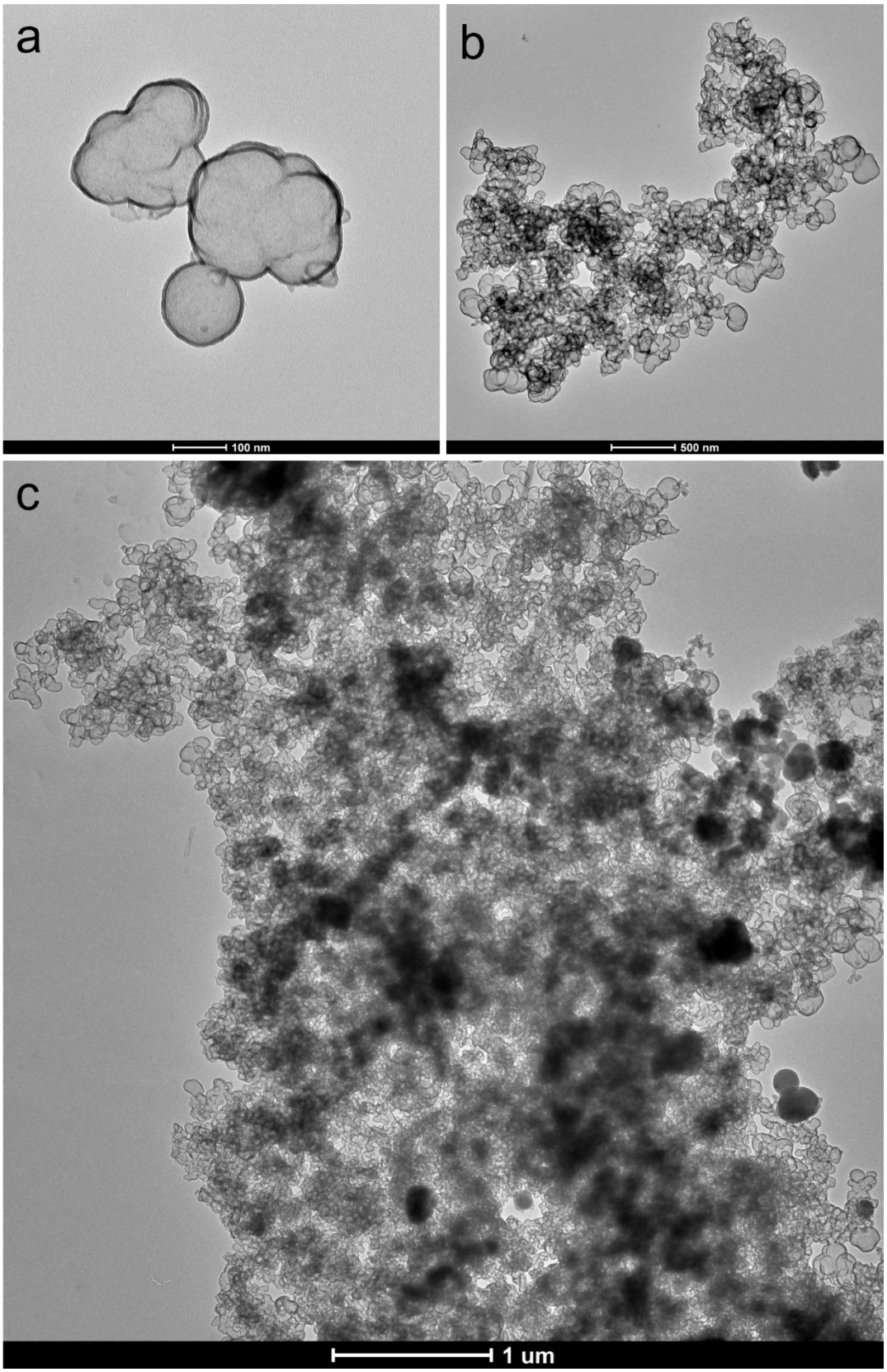

Figure S1. TEM images of silica compartments synthesized in the presence of Ca^{2+}.

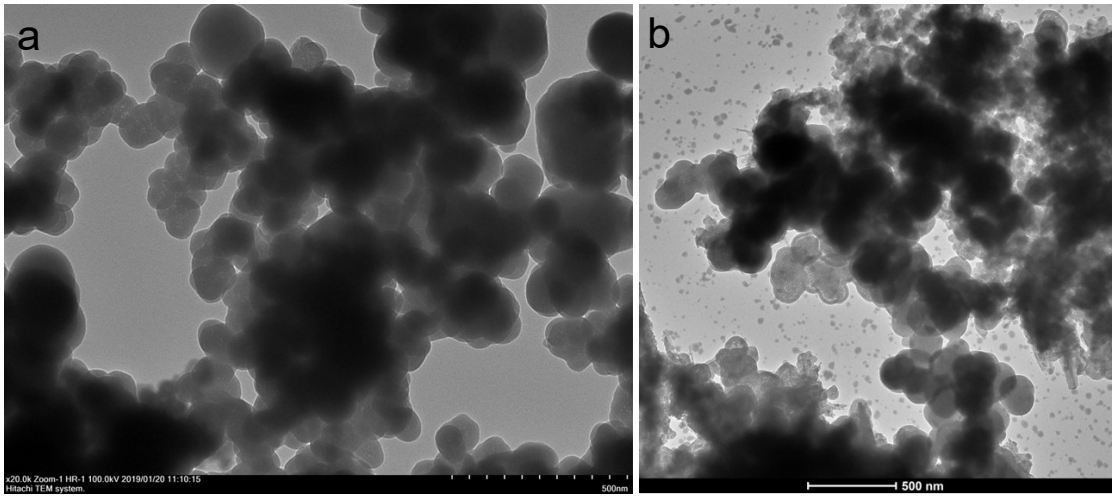

Figure S2. TEM images showing the solid silica clusters obtained by directly dropped the synthesis solution onto the TEM grid after incubation for 12 h.

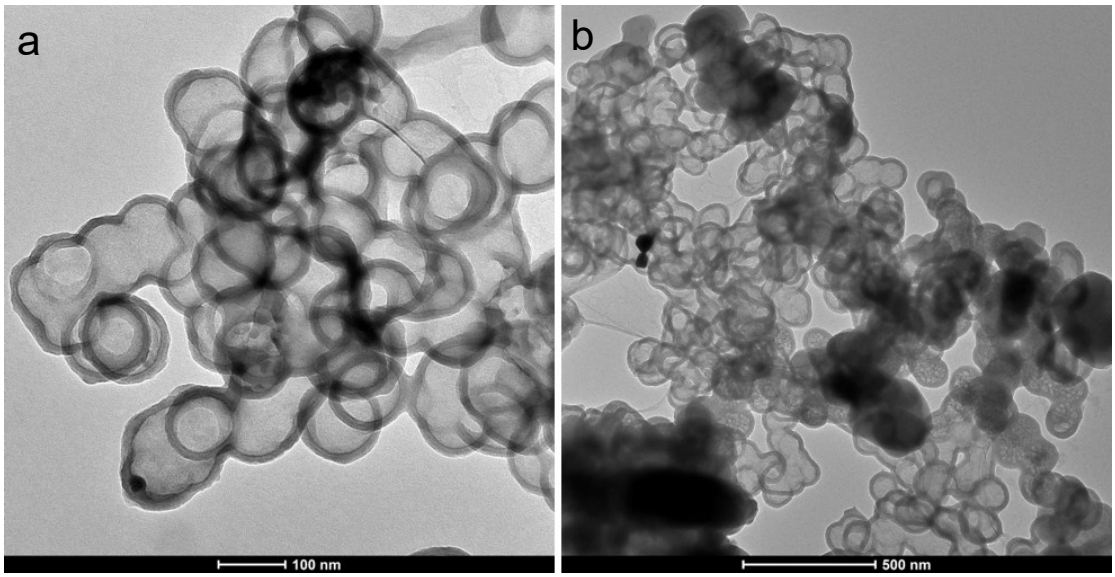

Figure S3. TEM images of silica compartments. The sample was obtained through diluting with water (7.5 times), being incubated for 7 h, and directly being dried on the TEM grid. This process didn't involve the centrifugation.

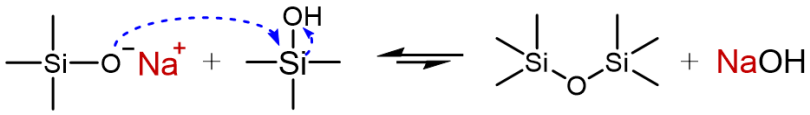

Figure S4. Chemical reaction equation illustrating the ion pairing of [–SiO⁻Na⁺] inhibits the cross-linking of poly(silicic acid) chains in alkaline synthesis solution.

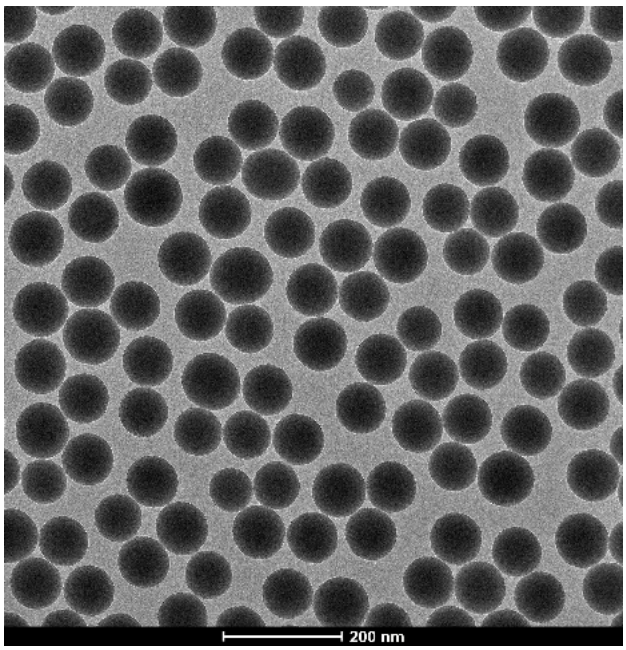

Figure S5. TEM image showing monodispersed silica spheres obtained in the absence of $CaCl_2$.

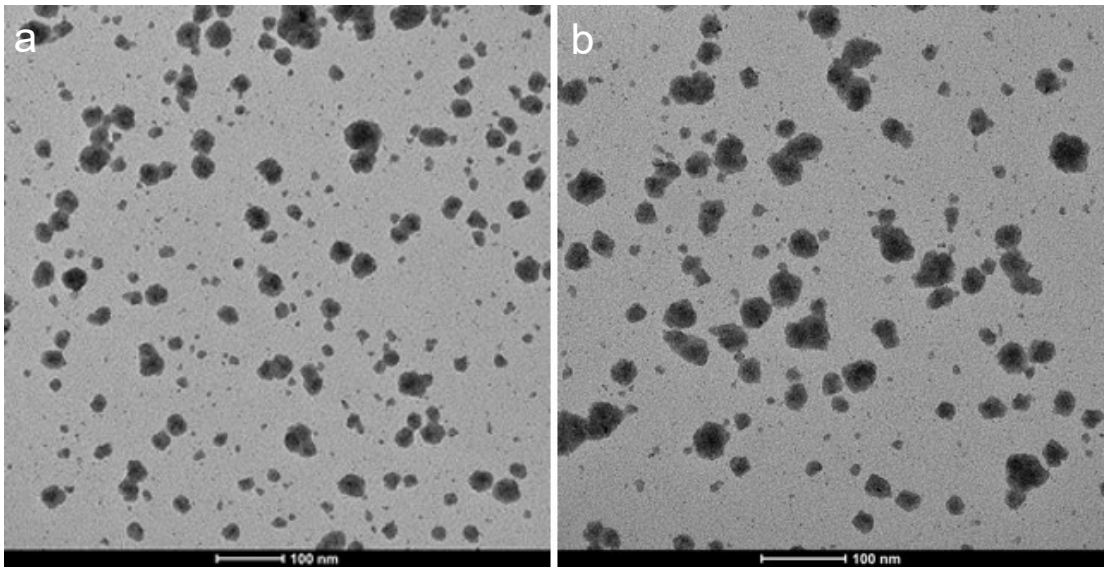

Figure S6. TEM images of the intermediates trapped at 20 min.

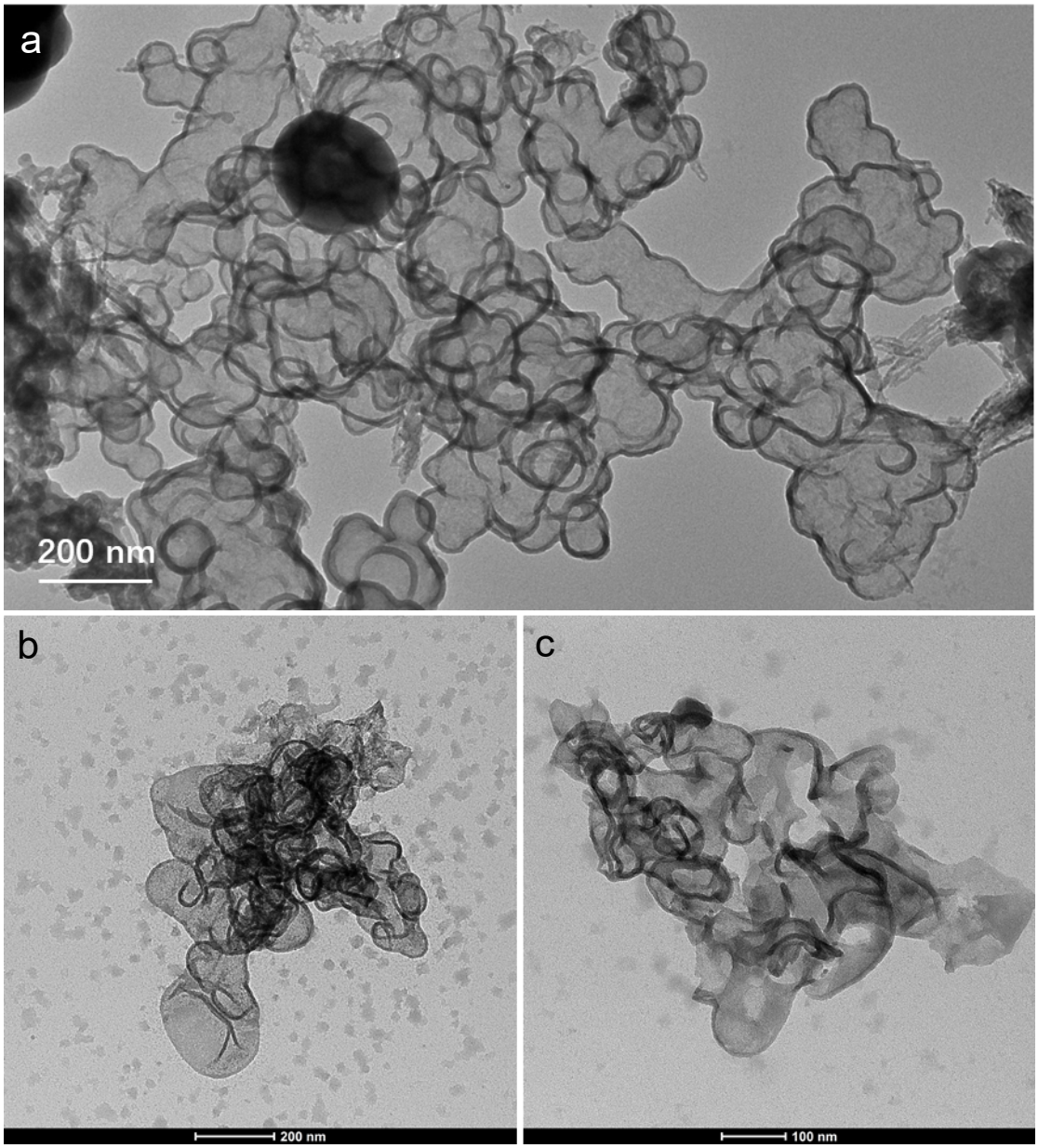

Figure S7. TEM images showing silica compartments obtained at $CaCl_2$ concentration of (a) 4mM and (b and c) 6 mM.

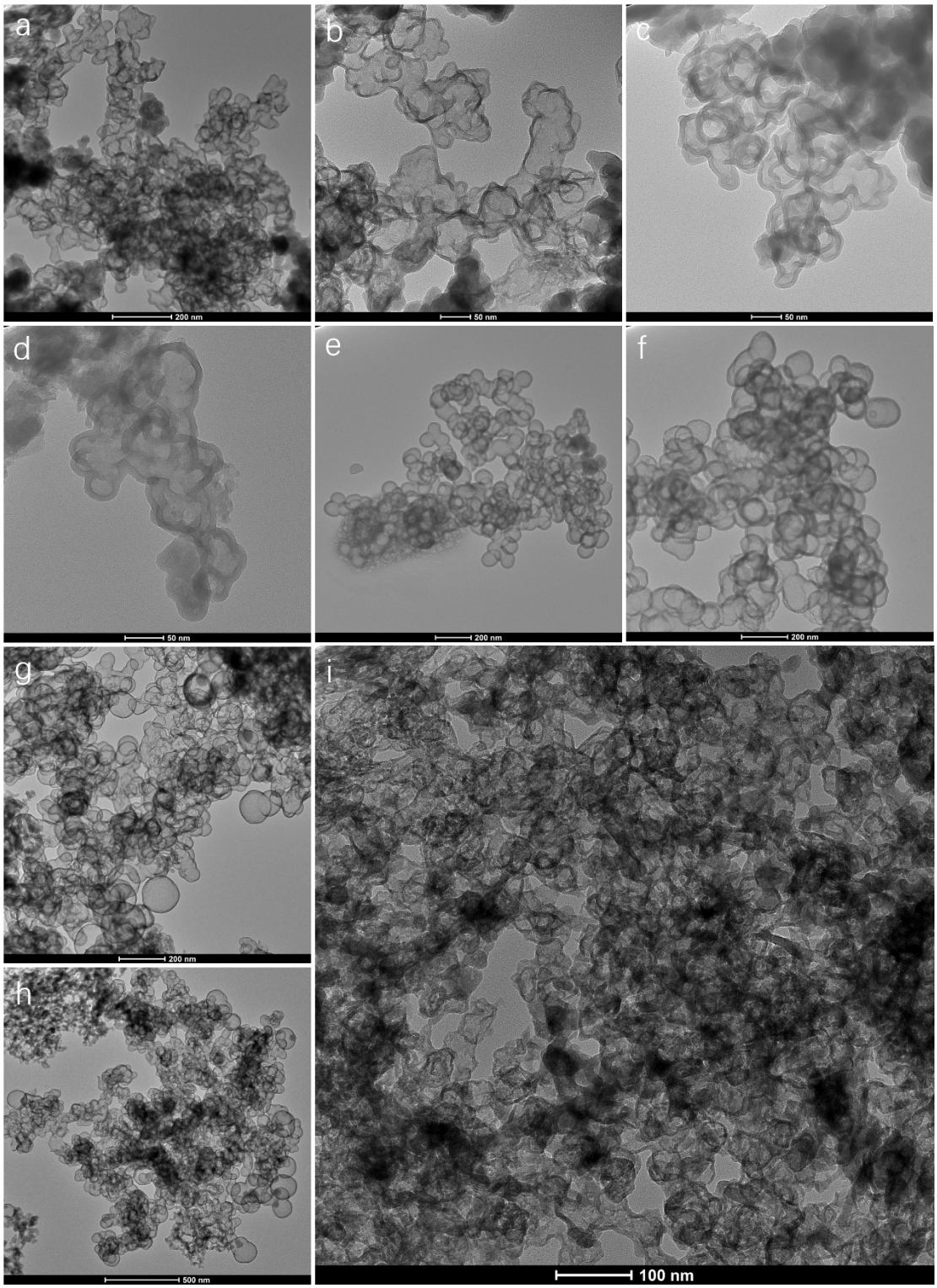

Figure S8. TEM images showing silica compartments obtained in (a and b) a mixture of silica precursors (TMOS, TEOS, and TPOS), (c and d) a mixture of bases (NH_3, NaOH, and KOH), (e and f) a mixture of solvent (MeOH, EtOH, IPA, and H_2O), (g and h) a mixture of salts ($CaCl_2$, $CuCl_2$, and $FeCl_3$), or (i) a mixture of all chemicals mentioned above.

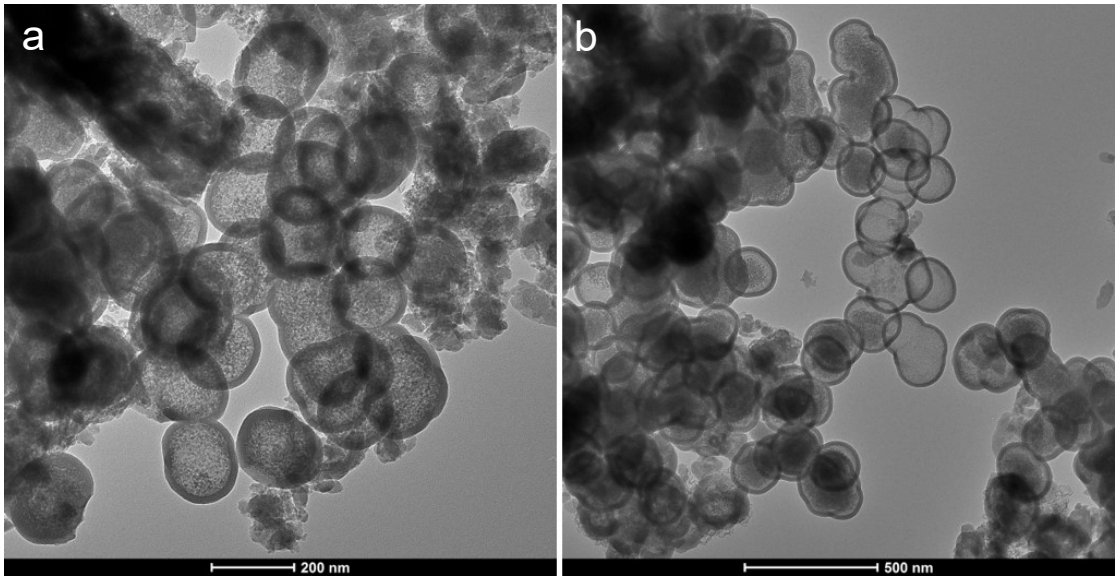

Figure S9. TEM images showing the product obtained when silica was synthesized under the condition of solvent evaporation and the as-obtained precipitate was re-dispersed in water.

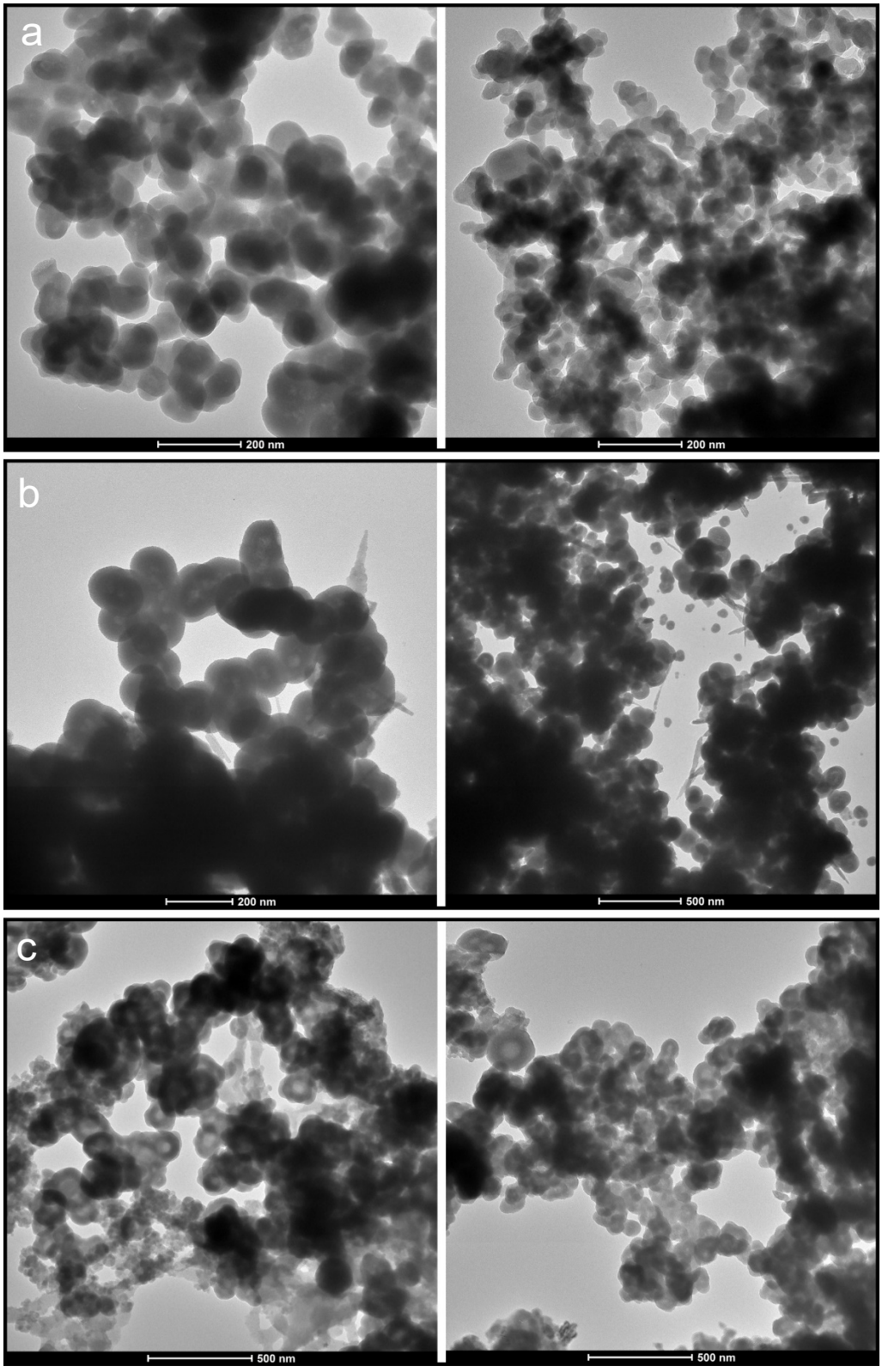

Figure S10. TEM images showing the coprecipitate of (a) silica and DNA, (b) silica and GOD, (c) silica and BSA.

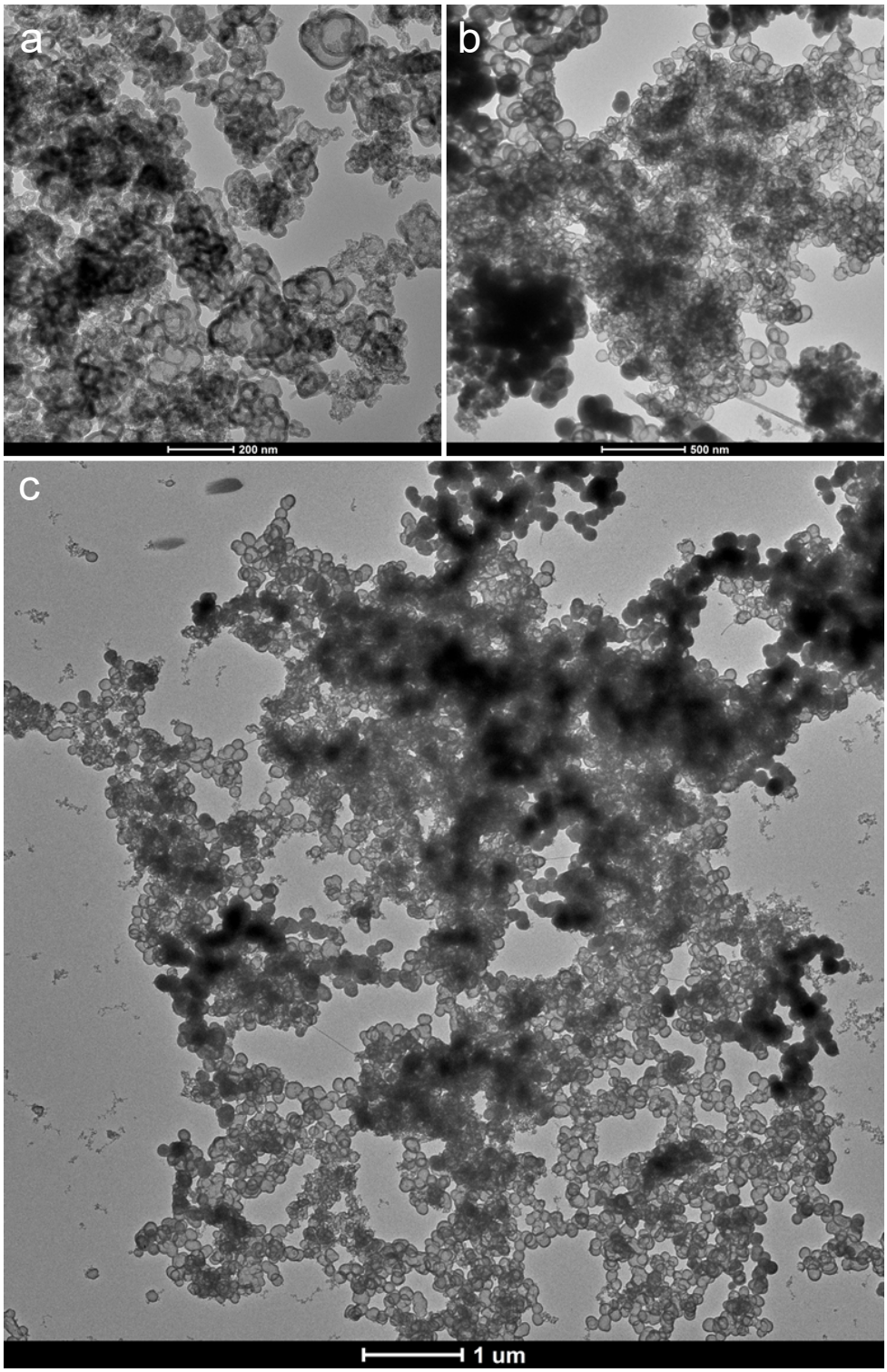

Figure S11. TEM images showing the silica compartments with enriched DNA.

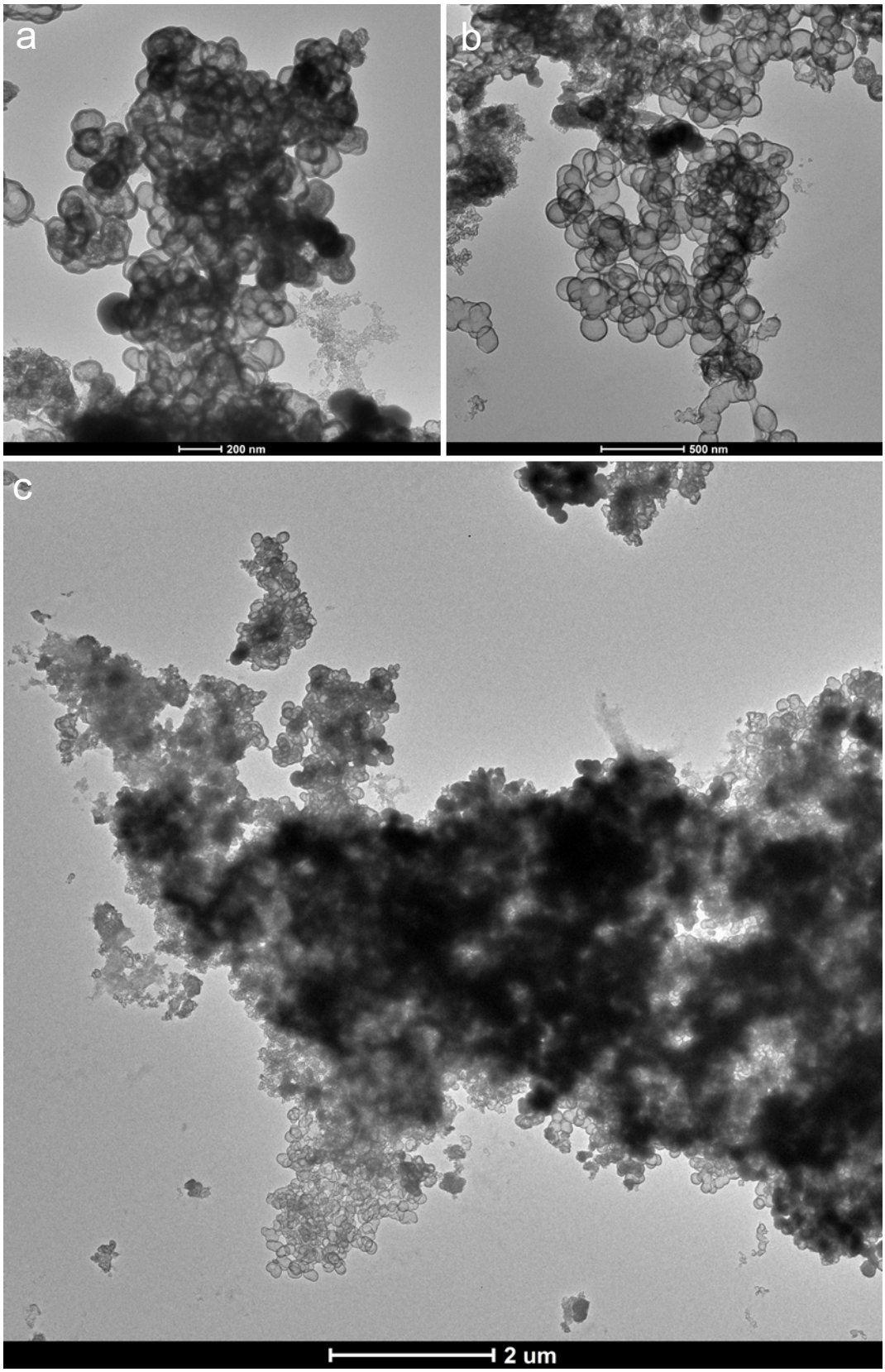

Figure S12. TEM images showing the silica compartments with enriched GOD.

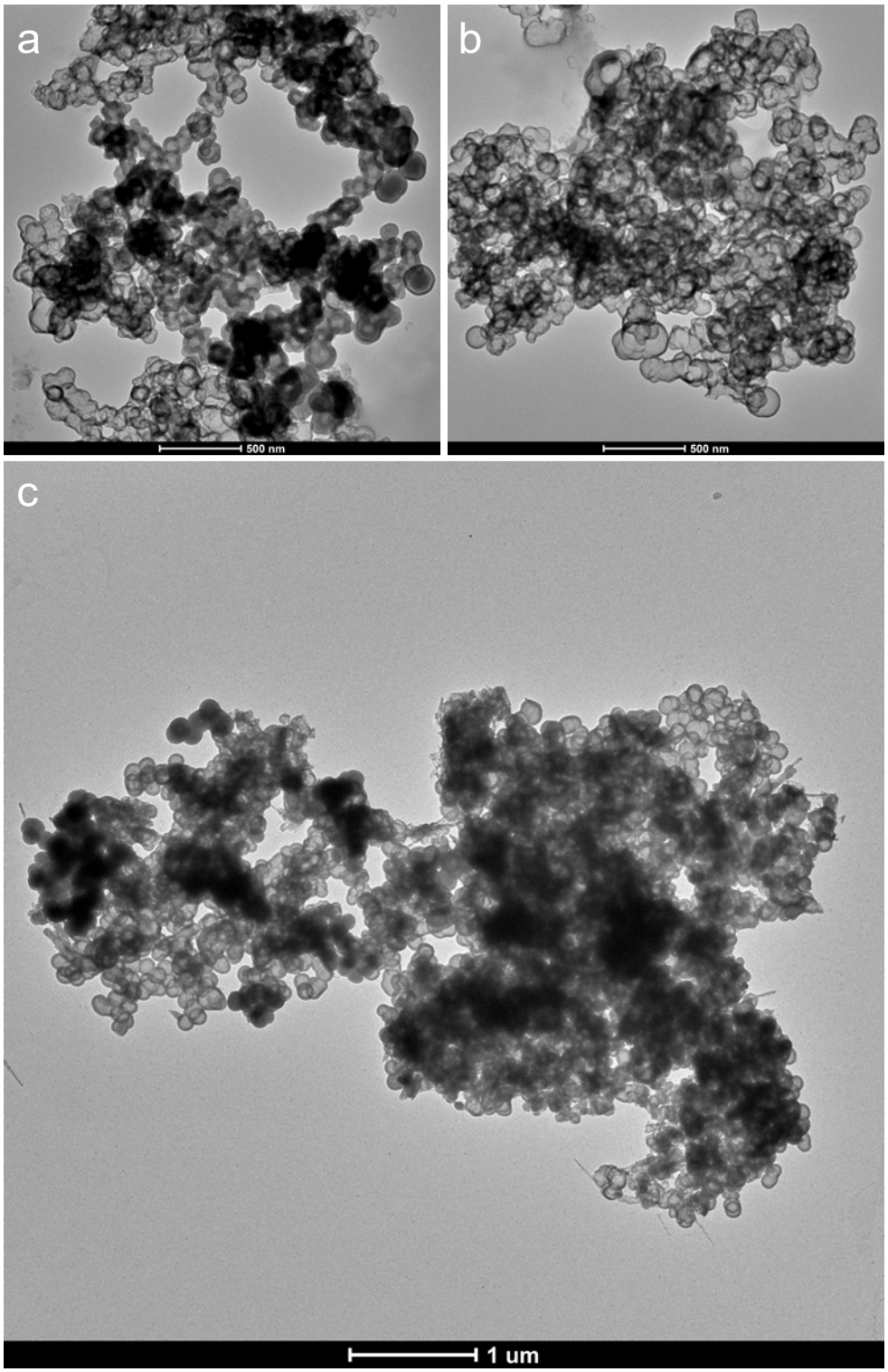

Figure S13. TEM images showing the silica compartments with enriched BSA.

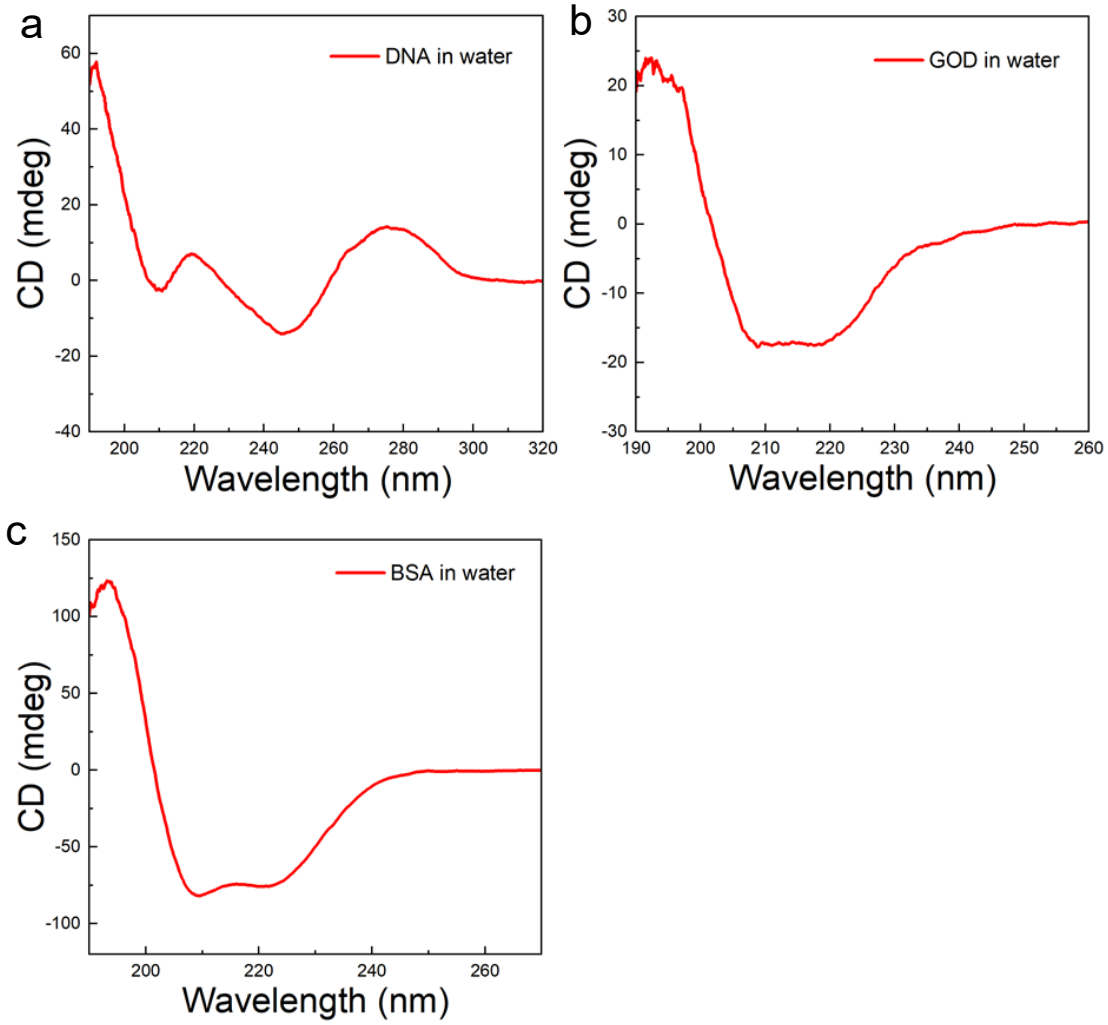

Figure S14. The CD spectra of free (a) CT-DNA, (b) GOD and (c) BSA in water.

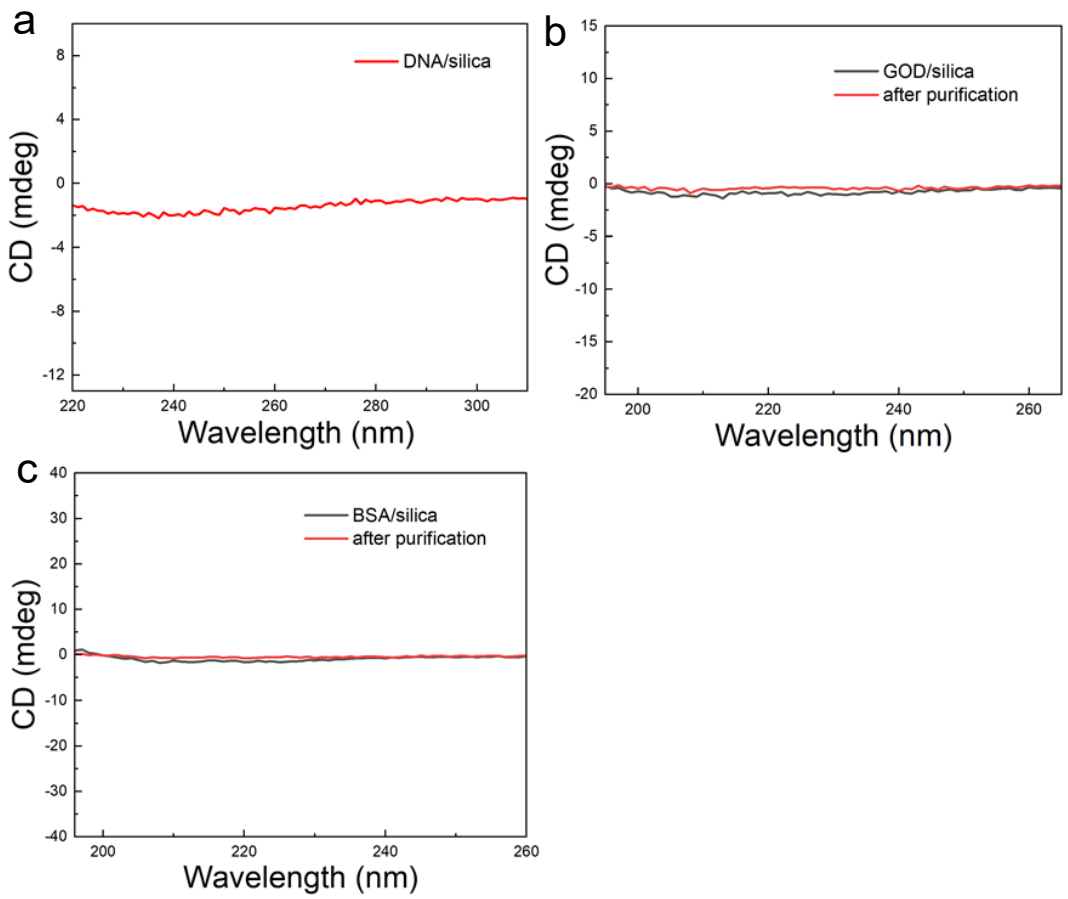

Figure S15. Silica NPs were incubated in DNA, GOD or BSA solution for 12 h to test the adsorption of biopolymers on silica surface. The corresponding CD spectra of isolated silica NPs with (a) DNA, (b) GOD or (c) BSA were shown.

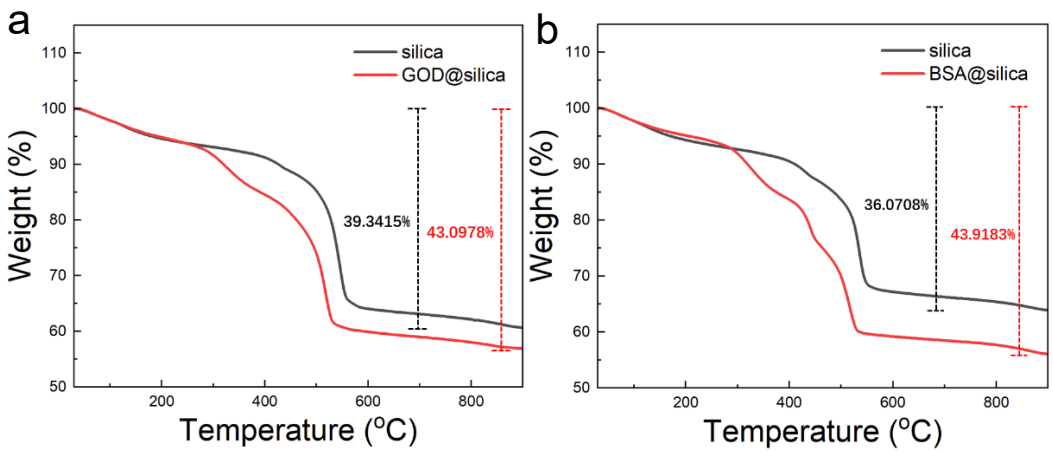

Figure S16. TGA of (a) GOD@silica and (b) BSA@silica and the corresponding plain silica. (These two figures are same as the Figure 5a and b in the main text)

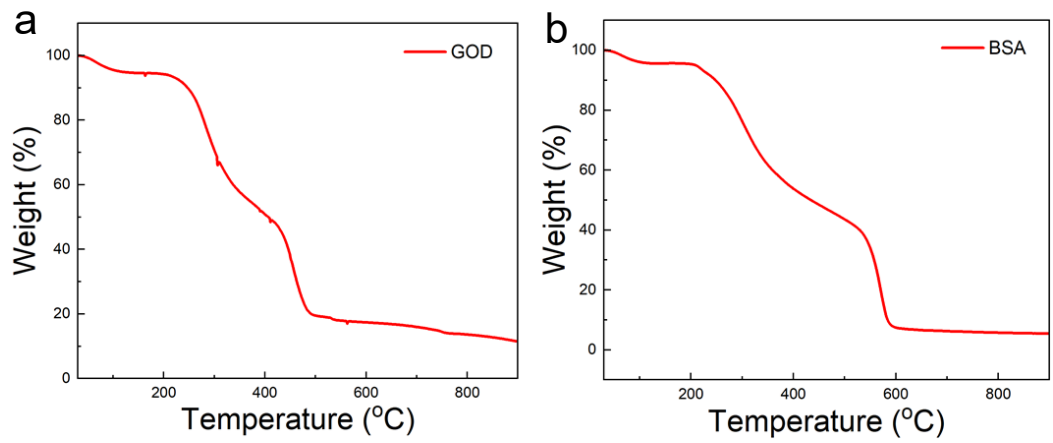

Figure S17. TGA of neat (a) GOD and (b) BSA.

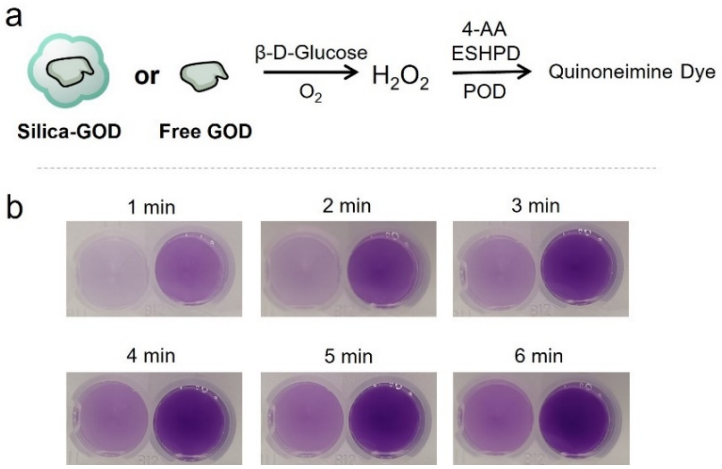

Figure S18. To illustrate that GOD remains catalytic activity after being trapped in silica compartments. (a) Reaction scheme for assaying the enzyme activity of GOD. (b) The sample of GOD@silica (left) or free GOD (right) was added into the assay mixture to produce the colored product. The color change captured from 1 to 6 minutes reflects the activity of the enzyme.

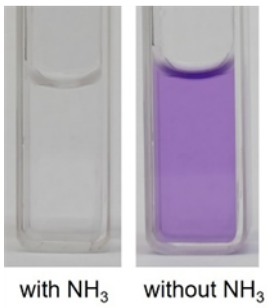

Figure S19. The free GOD was incubated with (left) or without NH_3 (right). The base treatment (0.3 M NH_3, 12 h) is same with the condition in silica synthesis. Then the enzymic activity was assayed using the 4-aminoantipyrine method, and the color change at 6 min was captured.

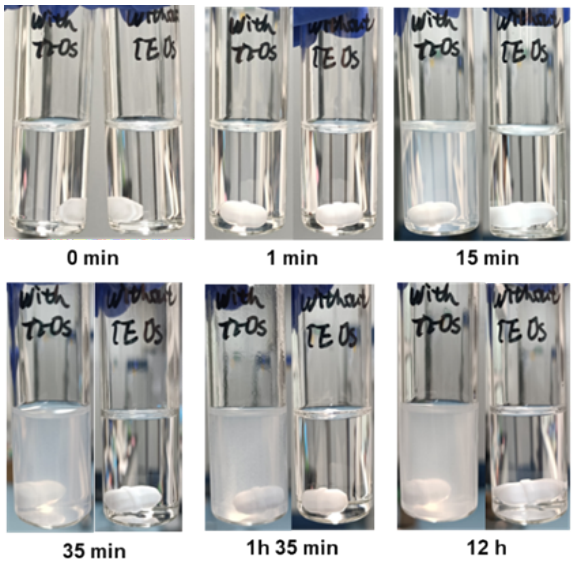

Figure S20. Digital photos showing the mixture with (left) and without (right) the addition of TEOS. Under the same conditions as the silica synthesis, in the absence of TEOS, the solution remained clear and transparent at all time without noticeable $Ca(OH)_2$ precipitates. In contrast, with the addition of TEOS, the solution quickly changed from transparent to turbid white within 15 minutes, indicating the nucleation and growth of silica. The results indicated that the initial precipitates captured at 20 min (Fig. S6) were Ca-doped silica, rather than $Ca(OH)_2$. Such investigation ruled out the possibility that $Ca(OH)_2$ as the template for the formation of silica compartments.