Supplementary Information (SI) for Soft Matter.

Biomimetic Mineralization of Positively Charged Silica Nanoparticles Templated by Thermoresponsive Protein Micelles: Applications to Electrostatic Assembly of Hierarchical and Composite Superstructures

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SUPPLEMENTARY METHODS

DNA manipulation and protein purification

An ELP gene encoding 96 repeats of the VPGVG sequence was amplified from Addgene plasmid pET25b(+)-V96 (number 68392) as a template. Primer pair 5'ATTCGAGCTCCGTCGACAGGCTTG3' and 5'TCGGATCCTGAAGATCATTATCAAAGCTTACCTACACCC3' introduced a unique HindIII site at the 3' end of the gene to enable modular insertion of solid-binding peptides at the C terminus of the gene product. Non-specific priming to repeated oligonucleotides regions in the V96 template resulted in amplification products of variable lengths. A product encoding 54 repeats of VPGVG followed by a KLGGGS flexible linker specifying a HindIII restriction site was recircularized yielding pET25b(+)-V54. To create a silica-binding derivative, the V54 gene was excised by NdeI-HindIII digestion and ligated into the same sites of pET24a(+)-sfGFP(G51C)-Car9,27 generating pET24a(+)-V54-Car9. An isogenic plasmid encoding the MG(VPGVG)54KLGGGS control polypeptide, pET24a(+)-V54, was constructed by digesting pET24a(+)-V54-Car9 with HindIII and XhoI and ligating the backbone with a DNA cassette obtained by hybridizing the 5'AGCTTGGCGGCGGCTCTTAATAAC3' and 5'TCGAGTTATTAAGAGCCGCCGCCA3' oligonucleotides. Plasmids transformed into E. coli Top10 were verified by sequencing and introduced into BL21(DE3) cells. For protein expression, overnight cultures of BL21(DE3) cells harboring pET24a(+)-V54 or pET24a(+)-V54-Car9 were grown at 37°C in 5 mL of LB medium supplemented with 50 μg/mL kanamycin and used to inoculate 250 mL of Terrific Broth (TB) supplemented with the same antibiotic. Cells were grown at 37°C without induction for 24 hours before cell paste was harvested by centrifugation at 4,000g for 20 min, resuspended in 25 mL of 20 mM Tris-HCl, pH 7.5, and disrupted by two cycles of

sonication for 9 min each (10s on, 20s off) at 30% amplification (Fisherbrand Sonic Dismembrator). Cell lysates were supplemented with 1 mL of 10% polyethylenimine (PEI), incubated on ice for 10 min, and centrifuged at 10,000g for 20 min at 4°C to remove insoluble materials and precipitated nucleic acids. ELPs were purified by inverse transition cycling (ITC).43 Each cycle started with ELP precipitation by adding 3 M of crystalline NaCl to the supernatant. After vigorous shaking, the mixture was rotated at 40 RPM for 30 min at room temperature and centrifuged at 4,000g for 20 min at 40°C. Soluble impurities were removed with the supernatant and the ELP-containing pellet was resuspended in 20 mL of pre-chilled deionized (DI) water and rotated as above at 4°C. Finally, the solution was centrifuged at 10,000g for 20 min at 4°C and the supernatant was subjected to one additional cycle of ITC. The purity of the final products was verified by Coomassie Blue-staining of SDS-PAGE minigels as shown in **Fig. S1.**

X-ray photoelectron spectroscopy (XPS)

XPS spectra shown in **Fig. S8** were taken on a Kratos Axis-Ultra DLD spectrometer with a monochromatized Al Kα X-ray and a low energy electron flood gun for charge neutralization. X-ray spot size is on the order of 700 x 300 μm. Pressure in the analytical chamber during spectral acquisition was less than 5 x 10^{-9} Torr. Pass energy for survey and detailed spectra (composition) was 80 eV. The take-off angle was 0° (0-degree take-off angle ~ 100 Å sampling depth). The Kratos Vision2 software program was used to determine peak areas and to calculate elemental compositions from peak areas. CasaXPS was used to peak fit the high-resolution spectra. For the high-resolution spectra, a Shirley background was used, and all binding energies were referenced to the C ls C-C bonds at 285.0 eV.

SUPPLEMENTARY FIGURES

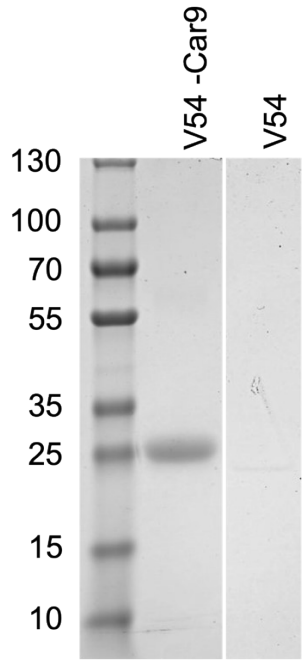


Figure S1 Coomassie Blue-stained SDS-PAGE of purified V54-Car9 and V54. Proteins were diluted to 100 μM and boiled in SDS before loading. The staining of V54 by Coomassie Blue is inefficient due to the lack of charged residues.

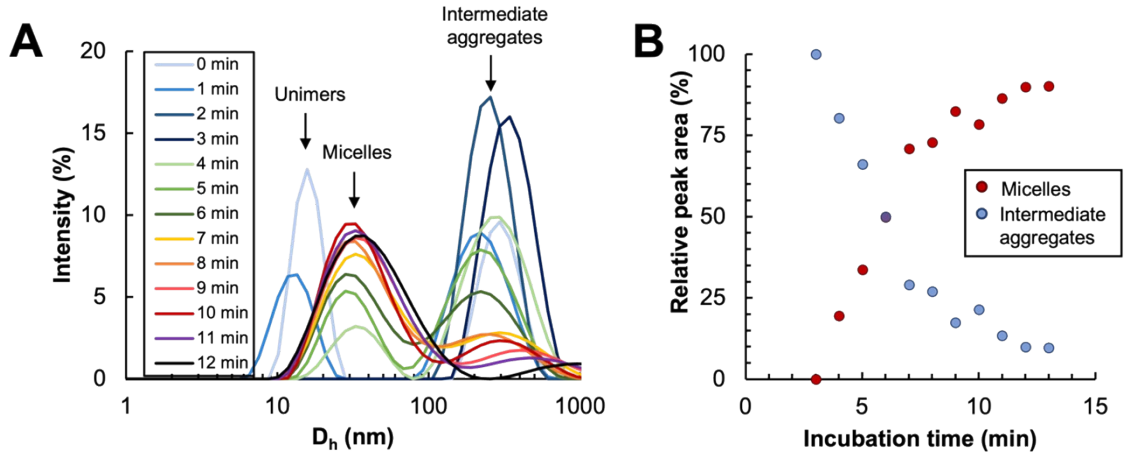


Figure S2 Kinetics of V54-Car9 micellization. (**A**) A 50 µM solution of V54-Car9 in water initially at room temperature was transferred to 65°C. DLS intensity profiles collected at the indicated time points show the transition of unimers to intermediate aggregates that progressively collapse into micelles. (**B**) Quantification of the evolution of intermediate aggregates (300 nm peak) and micelles (30 nm peak) over time.

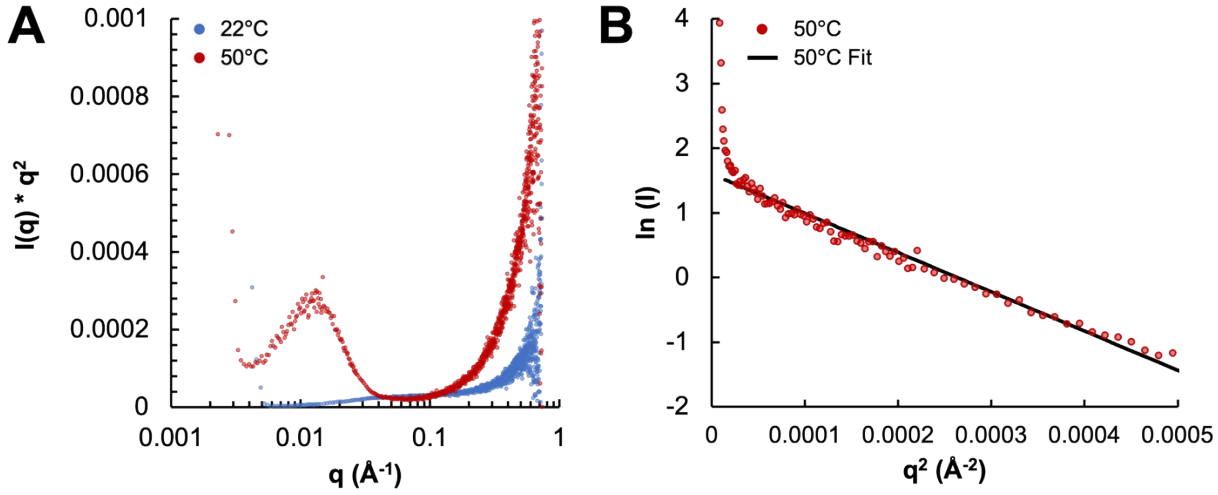


Figure S3 Transformations of SAXS data. **(A)** Kratky plot of SAXS data illustrates the degree of random coil and folded conformation for a 710 µM solution of V54-Car9 below (25°C, blue) or above (50°C, red) T_t. **(B)** Guinier plot and linear fit of low-q scattering intensity of V54-Car9 at 50°C.

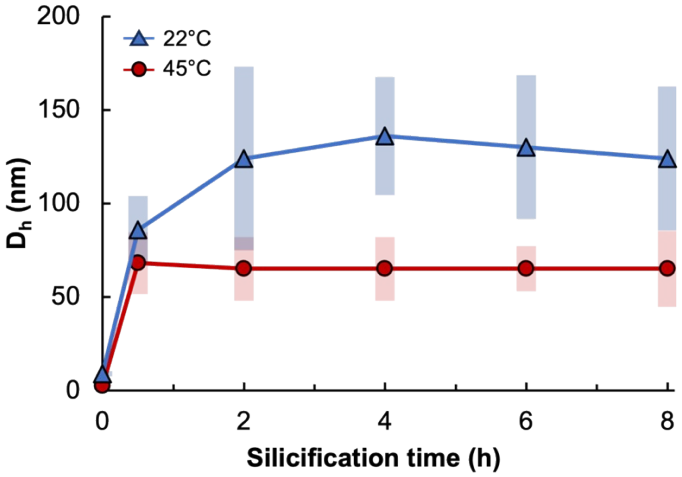


Figure S4 Kinetics of silica particle growth at 22°C (blue triangles) and 45°C (red circles). Bars correspond to the full width at half maximum (FWHM) of the intensity size distributions measured by DLS. Experiments were conducted with 75 µM of V54-Car9 and 100 mM of silicic acid.

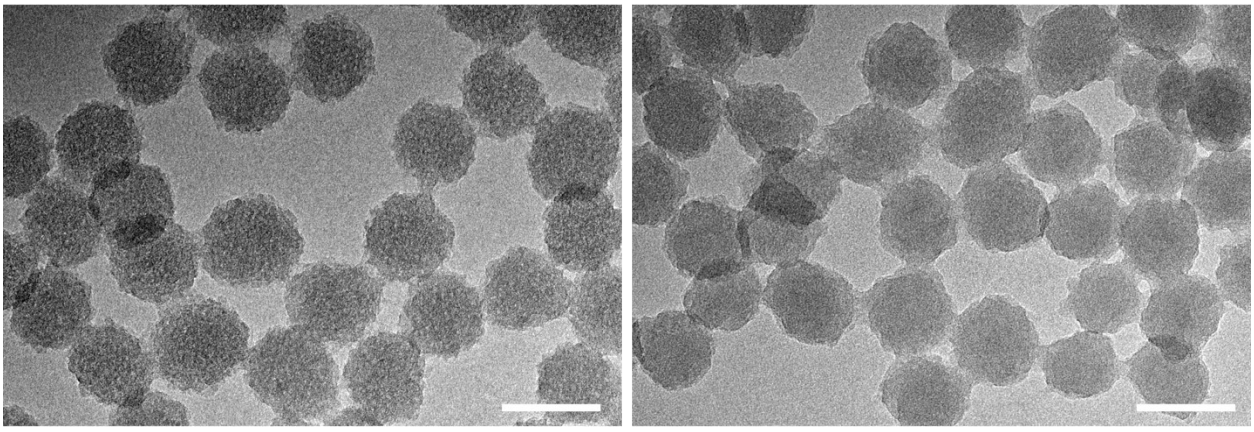


Figure S5 Additional field of view TEM images of mineralization products obtained by incubating 75 µM of V54-Car9 with 100 mM of silicic acid at 45°C for 8h. Scale bars are 50 nm.

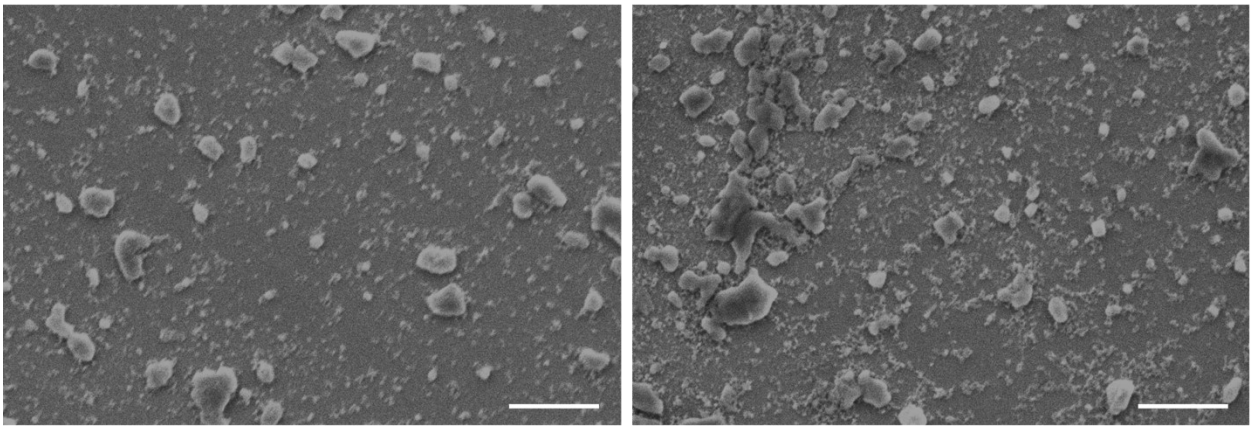


Figure S6 Representative field of view SEM images of mineralization products obtained by incubating 75 μM of V54 with 100 mM of silicic acid at 45°C for 8h. Scale bars are 500 nm.

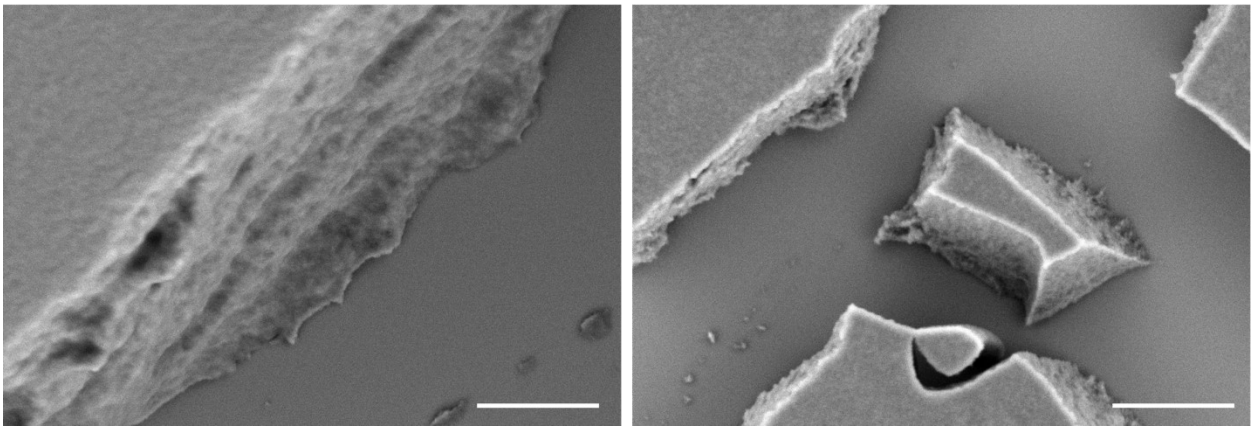
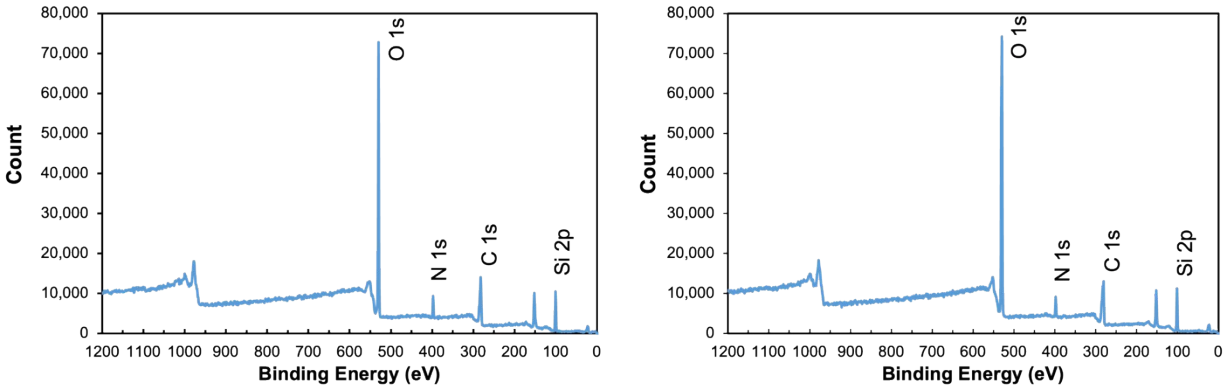


Figure S7 Representative field of view SEM images of mineralization products obtained by incubating 100 mM of silicic acid at 45°C for 8h. Scale bars are 1 μm.



Peak ID	B.E. (eV)	Atomic %
O 1s	530	42.6
C 1s	282	37.0
Si 2p	101	15.3
N 1s	398	5.2

Peak ID	B.E. (eV)	Atomic %
O 1s	530	39.9
C 1s	281	39.4
Si 2p	101	15.9
N 1s	398	4.9

Figure S8 XPS analysis of mineralization products obtained by incubating 75 µM of V54-Car9 with 100 mM of silicic acid in water at 45°C for 8h. Two different spots were sampled to yield a mean elemental composition of 41.3% oxygen, 15.6% silicon, 38.2% carbon, and 5.1% nitrogen.

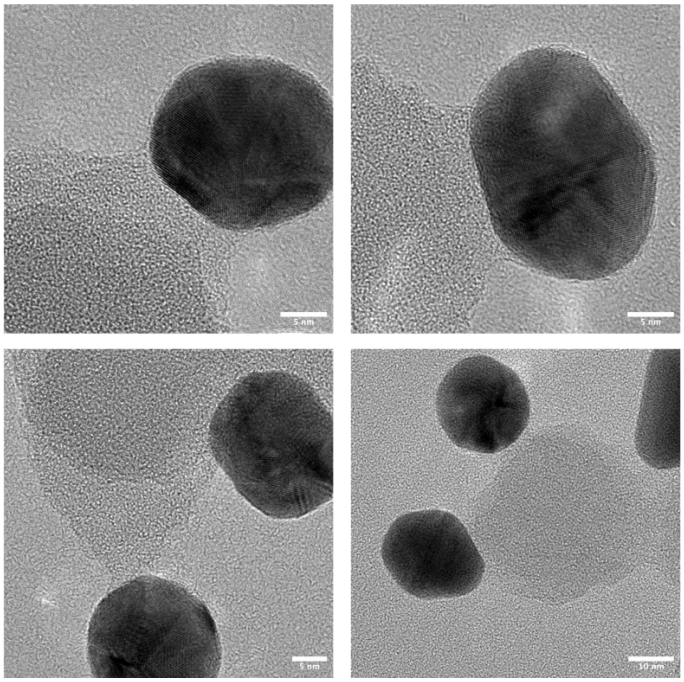


Figure S9 Additional HRTEM images of mineralized silica/Au co-assemblies.

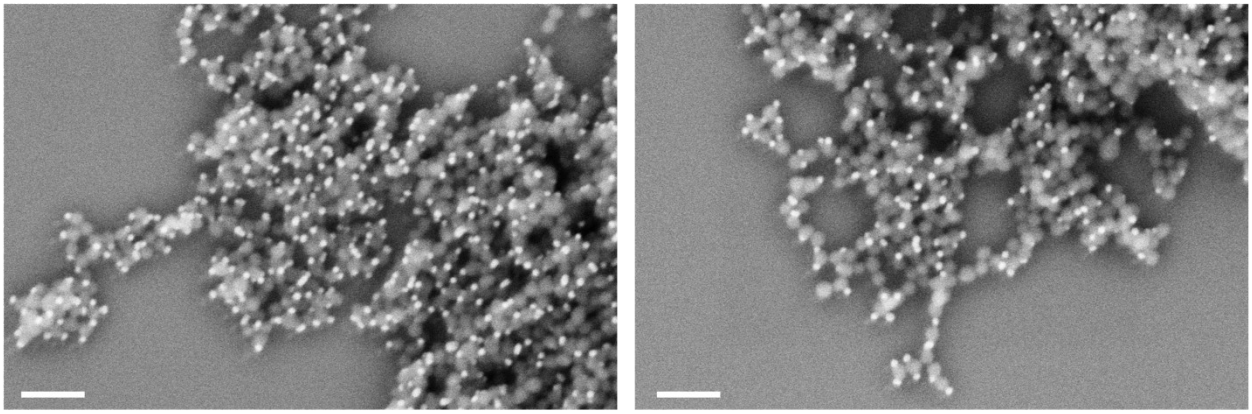


Figure S10 SEM images of mineralized silica/Au co-assemblies after 6 months of storage. Scale bars are 200 nm.