A nacre protein sequence compartmentalizes mineral polymorphs in solution. Supporting Information**

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Experimental Section

Polypeptide synthesis, purification, and sample preparation.

The 30-mer polypeptide n16N, representing the 1-30 AA N-terminal domain of n16, was synthesized at the 100 micromole synthesis level using the protocol described in our earlier work.[1] This peptide featured free amino termini and C^{alpha} amide "capping" to mimic its attachment to the protein and negate the charge contribution from the alpha-carboxylate C-terminus.

In situ potentiometric titration.

A computer controlled potentiometric titration system (Metrohm AG, Herisau, Switzerland) consisting of two titration devices (Titrando 905, controlling four dosing devices; Dosino 800, that operate a 807 Dosing Unit) are utilized for the titration measurements. The 807 Dosing Unit incorporates a 2 mL glass cylinder, whose depletion is divided into 10,000 steps resulting in a minimum doseable volume of 0.2 µL. One pH electrode and one calcium ion selective electrode were utilized to determine the amounts of H^+ and Ca^{2+} ions in the reaction buffer.

One dosimeter containing 10 mM NaOH was used to keep the pH of the 10 mM Carbonate reaction buffer (mixture of 10 mM Sodium Bicarbonate and 10 mM Sodium Carbonate) constant at pH = 9.75. The Ca^{2+} ion electrode was sensitive to 100 nM concentrations of Ca^{2+} ions in solution. Together with a second dosimeter containing a solution of 10 mM $CaCl_2$, the exact amount of Ca^{2+} ion concentration titrated into the carbonate reaction was controlled. Both the Ca^{2+} concentration and pH of the reaction buffer were measured as a function of time.

At the start of the titration experiments to observe the effects of sample biomolecules in solution on calcium carbonate mineralization, at stock solution of n16N (1 mg/mL or 267 ⌈M) in unbuffered deionized distilled water (UDDW) was added to the reaction buffer. The final concentration of the n16N investigated were 0.1, 1.0, 10, and 100 µg/mL. This concentration range is 5 – 100 times lower than what was previously utilized in earlier n16N mineralization and oligomerization experiments.[1-3] The titration of $CaCl_2$ occurred at a rate of 0.01 mL/minute until a volume of 15 mL of $CaCl_2$ was added to the reaction buffer.

Visualization of titrant precipitates.

A Zeiss ImagerM2.m (Carl Zeiss MicroImaging GmbH, Jena Germany) with polarized light filters and computer controlled X-Y-Z sample stage using Zeiss AxioVision 4.8.2 (Carl Zeiss MicroImaging GmbH, Jena Germany) was used to examine the structural features of the solid phase from the titration reactions at the micrometer length-scale. For smaller length scales, SEM and TEM imaging were performed. For SEM, precipitants in the titrant were filtered with 200 µm pore sizes, air dried, washed with ethanol and placed onto double sided carbon tape for microstructural analysis. A desktop SEM system (Hitachi TM-3000, Hitachi High-Technologies Europe GmbH, Krefeld Germany) at 15 kV under backscatter electron microscopy mode was used to examine the morphology of calcium carbonate crystals (solid fraction) found in solution. For TEM, Samples from the titration measurements were resuspended in 50%, 70%, and 100% solutions of analytical grade ethanol (AnalaR Normapur, VWR Internation S.A.S, Fontenay-sous-Bois France) and spotted onto 400 mesh copper grids coated with carbon film (Quantifoil Micro Tools GmbH, Jena Germany). TEM micrographs were imaged with an in lens column filter Zeiss Libra 120 (Carl Zeiss SMT, Oberkochen Germany). Each sample was observed at 120 mV with 1 mrad at magnification series (8-100 kX) to examine micro- and nano-structures of each sample.

ATR-FTIR

A standard ATR-FTIR spectrophotometer (Spectrum 100, Perkin-Elmer Life and Analytical Sciences, Bridgeport, CT, USA) was used to obtain spectra between 4000 and 650 cm−1 for each sample. The spectra were analyzed with a spectrum analysis program (Spectrum version 6.2.0, Perkin-Elmer Life and Analytical Sciences, Bridgeport, CT, USA) supplied by the FTIR manufacturer.

XRD

A powder X-ray diffractometer (D8 Advance, Bruker AXS GmbH, Berlin, Germany) was used to measure the crystalline reflections of the samples with a scan time of 1 h each and a scan range of $2\theta \approx 10-80°$.

Detection of solution-suspended polypeptide – mineral deposits.

In previous mineralization studies of n16N, the emphasis was on sampling of deposits which collected at the bottom of mineralization assay wells.[1-3] In this report, we extend our observations to n16N-generated mineral deposits which are suspended in assay solutions and do not settle out during the assay. The former mineralization assays are single stage and use solid $(NH_4)_2CO_3$ that vaporizes over time (16

hrs, 15 °C) and yields carbon dioxide gas which subsequently, dissolves into the assay solution (8, 16, 19, 25). Dissolution of CO_2 produces CO_3^{2-} since the pH raises by dissolution of NH_3 from $(NH_4)_2CO_3$.[1-6] The initial pH of the assay solutions containing n16N peptide and 12.5 mM $CaCl_2$ was found to be 3.3, and reached a value of 8.0 – 8.3 at the conclusion of the assay. These assay solutions contained either no peptide (negative control) or final assay concentrations of 50, 75 and 100 micromolar n16N as employed in previous mineralization studies.[1-3] Using supernatant sampling[5,6] we recovered mineralization clusters from negative control (no n16N added) and n16N-containing assay solutions for TEM visualization and electron diffraction.

For TEM studies, an aliquot (~ 10 microliters) of the assay supernatant was removed and carefully spotted on a Au TEM grid (coated with a Formvar layer that is stabilized by carbon; Ted Pella, Inc). This spot was gently washed with ethanol and deionized distilled water. This spotting and washing procedure was repeated again using a fresh aliquot each time, such that enough insoluble assay material would be deposited on the grids for subsequent visualization. Once sufficient aliquot transfers to the grids were completed, the grids were allowed to air dry. TEM imaging and electron diffraction measurements were performed on grids using a Philips CM12 transmission electron microscope at 120 kV. Cropping of TEM images and adjustment of brightness/darkness and contrast levels were performed using Adobe Photoshop (Adobe Systems Inc., Menlo Park, CA USA).

CD Spectroscopy with varying pH

Solutions for measuring the circular dichroism (CD) spectra were prepared as 0.6 ml samples in 10 mM carbonate buffer. CD experiments were performed on a Jasco 715 spectropolarimeter (Jasco Hachioji, Tokyo, Japan) with a Peltier temperature control unit. Using 1 cm quartz cuvettes, the spectra were recorded at 0.5 nm intervals between 190 and 240 nm at 20°C, using a 50 nm/sec scan rate. The spectra were corrected by buffer background subtraction and smoothened using the Savitzky Golay algorithm. Data analysis was performed by using an online evaluation software (K2D3).

Supplementary Figures

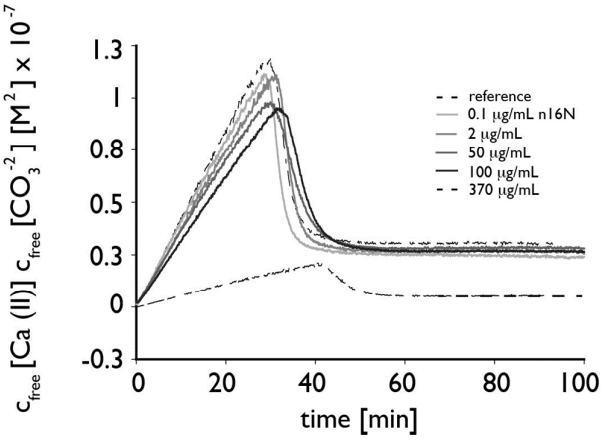

Figure S1: Solubility products for Ca^{2+} titrations in the presence of varying concentrations of n16N.

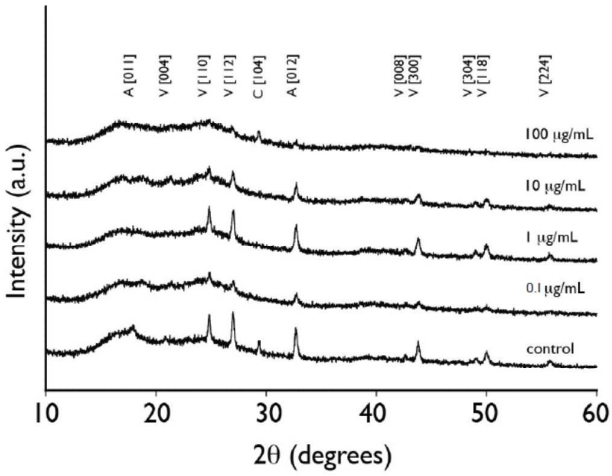

Figure S2: Powder x-ray diffraction spectra of titration deposits obtained from n16N samples and reference (i.e., negative control with no peptide added).

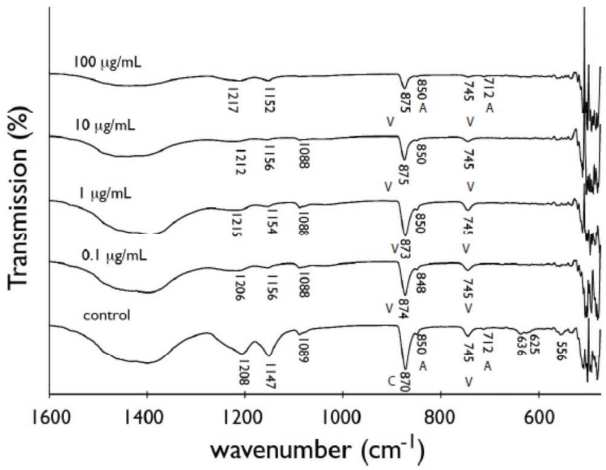

Figure S3: FT-IR spectra of titration deposits obtained from n16N samples and reference (i.e., negative control with no peptide added).

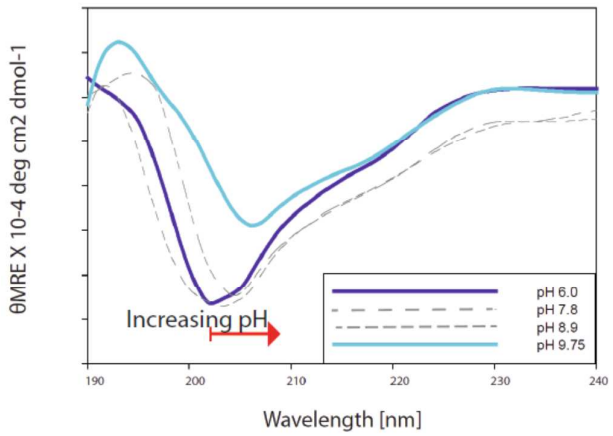

Figure S4: The effects on n16N secondary structure from pH variations as observed by CD spectroscopy

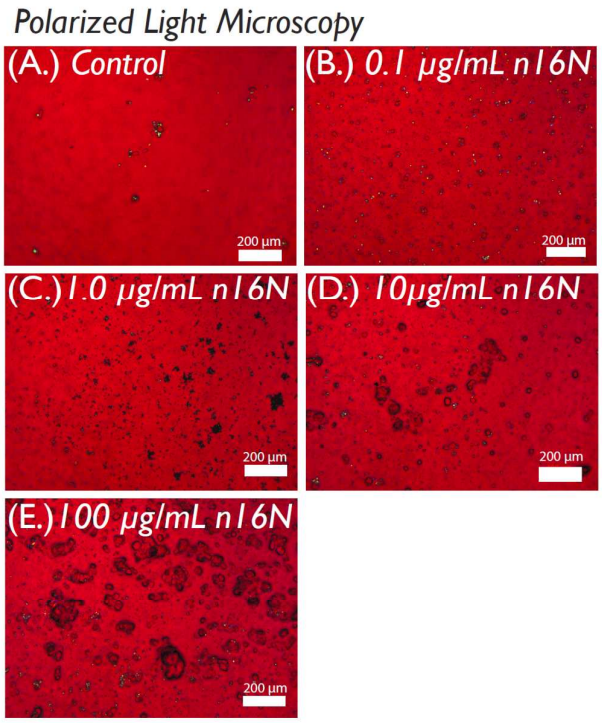

Figure S5: Polarized light microscopy of the mineral phase with varying concentrations of n16N and without n16N present during mineralization.

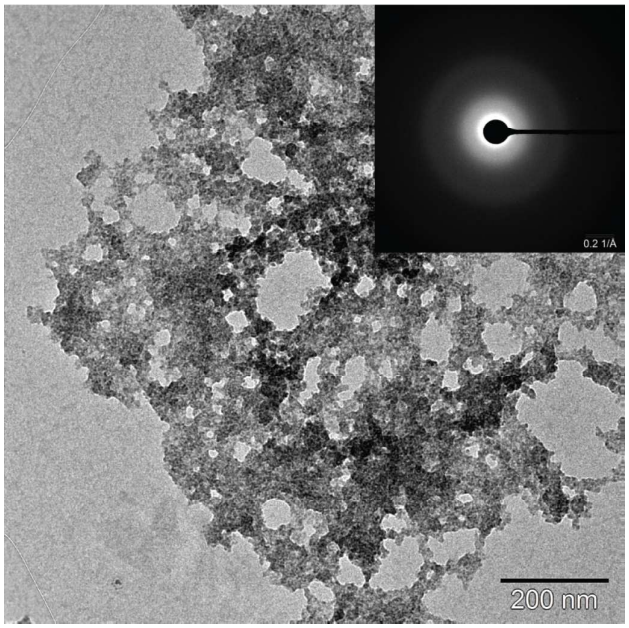

Figure S6: TEM micrograph and electron diffraction of full-length n16 assemblies shown to resemble that of n16N self-assembled localized compartments (as shown in Figure 3).

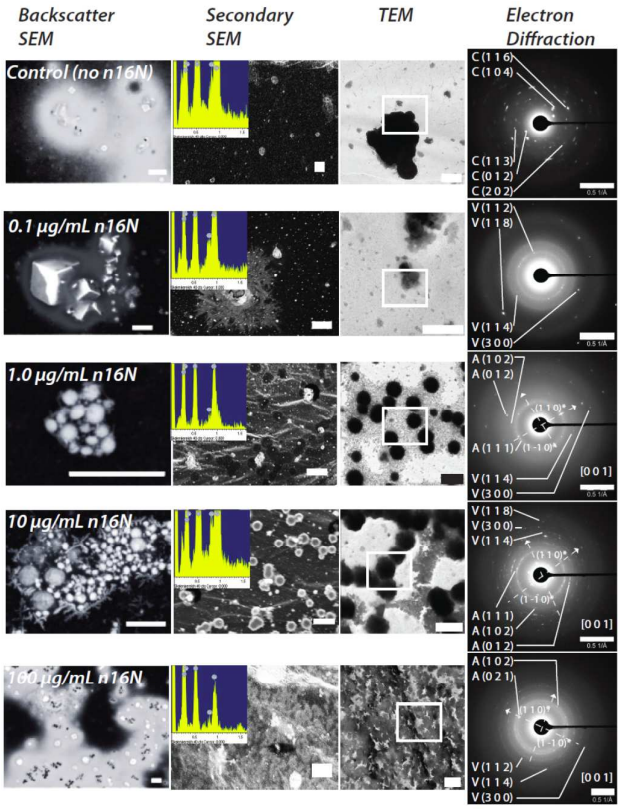

Figure S7: SEM and TEM micrographs along with corresponding electron diffraction patterns of $CaCO_3$ products occurring from the presence of varying concentrations of n16N during mineralization.

Table S1. Observed FTIR Data for Figure S3.

Sample	Aragonite[a]	Calcite[a]	Vaterite[a]
Control	850, 712	870, 713	870, 745
0.1 µg/mL	---	---	870, 745
1 µg/mL	---	---	870, 745
10 µg/mL	---	---	870, 745
100 µg/mL	850, 712	--	870, 745

[a] Units are in [cm^{-1}].

Table S2. Circular dichroism spectroscopy of n16N under various pH conditions Under increasing pH environments, CDS results indicate n16N undergoes beta strand to alpha helical structure transitions as shown. Evaluation of CDS data was performed with K2D3.

pH	α helical [%]	β strand [%]
6.0	45.47	2.54
7.8	49.81	1.25
8.9	59.47	0.43
9.75	55.98	1.48

Reference

[1] F.F. Amos, C.B. Ponce, J.S. Evans, *Biomacromolecules* **2011**, *12*, 1883-1890.
[2] R.A. Metzler, *et al. J. Am. Chem. Soc.* **2010**, *132*, 6329-6334.
[3] E. Keene, J.S. Evans, L. Estroff, *Crystal Growth and Design* **2010**, *10*, 1383-1389; E. Keene, J.S. Evans, L. Estroff, *Crystal Growth and Design* **2010**, *10*, 5169-5175.
[4] C.B. Ponce, J.S. Evans, *Crystal Growth and Design* **2011**, *11*, 4690-4696.
[5] F.F. Amos, J.S. Evans, *Biochemistry* **2009**, *48*, 1332-1339.
[6] F.F. Amos, M. Ndao, C.B. Ponce, J.S. Evans, *Biochemistry* **2011**, *50*, 8880-8887.