Supporting Information:

Extended Experimental Methods and Materials

Solvents and reagents were purchased from Fisher Scientific and Sigma-Aldrich respectively, while amino acids and resins used in peptide synthesis were provided by NovaBiochem (San Diego, CA) and Applied Biosystems (Foster City, CA). Culture medium was purchased from Invitrogen, Inc (Carlsbad, CA). Fetal bovine serum was obtained from Hyclone, Inc (Logan, UT). All materials and reagents for electron microscopy were purchased from Electron Microscopy Sciences (Fort Washington, PA).

The synthesis of the peptide portion of the molecule was performed using standard solid phase synthesis on an Applied Biosystems 433A automated peptide synthesizer. The peptide was grown on an aspartic acid-functionalized Wang polystyrene resin, using 0.95 molar equivalents of HBTU and 6 equivalents of diisopropylethylamine (DIEA) for each new amino acid coupled to the resin. A C_{16} alkyl tail was subsequently added to the N-terminus of the peptide manually, by adding 3 molar equivalents of palmitic acid to the peptide, in the presence of 0.95 palmitic acid molar equivalents HBTU and 12 peptide molar equivalents of DIEA. The peptide amphiphile was then cleaved from the polystyrene resin and amino acid side groups were deprotected in 95% trifluoroacetic acid (TFA), 2.5% triisopropylsilane (TIS), 2.5% deionized water. TFA was removed in a rotary evaporator and the peptide was collected by precipitation in cold diethyl ether. Filtered product was dried and then redissolved at 10 mg/mL in slightly basic water (pH = 7.5-8) and distributed in 1 mL aliquots. At this stage the

replacement of trifluoroacetate bound to positively charged residues was achieved by treating these aliquots with 100 mL of 0.1 M hydrochloric acid (HCl). These aliquots were lyophilized and stored at –30°C. Electrospray Ionization Mass Spectrometry performed on solutions diluted to 1 mg/mL was used to confirm the peptide sequence.

Lyophilized PA was reconstituted at 10 mg/mL in deionized water and 50 μL of this clear solution were delivered to individual wells of 8-well chamber slides. 50 μL of calcium-supplemented culture medium (MEM-α, containing 20 mM $CaCl_2$, 10% bovine serum albumin, 1% penicillin/streptomycin, and 3 mM β-glycerophosphate (unless otherwise specified)) were mixed with the solutions taking care not to disrupt newly forming gel. Collagen control samples were prepared using 3 mg/mL soluble type I bovine collagen (Vitrogen: Cohesion Technologies, Inc., Palo Alto, CA). The collagen solution was mixed in equal volumes with the culture medium (MEM-α, 10% bovine serum albumin, 1% penicillin/streptomycin, and 3 mM β-glycerophosphate) and incubated for 5 minutes at 37°C. 100mL of this mixture were then placed in individual wells of 8-well chamber slides where they were gelled by incubation at 37°C for 2 hours. Alginate gels were prepared using 2% sodium alginate from brown algae (Sigma-Aldrich), dissolved in hank's balanced salt solution. 50 μL of this solution were delivered to individual wells of 8-well chamber slides. Gelation was achieved by addition of 50 μL of alginate gelation medium (MEM-α, supplemented with 170 mM $CaCl_2$, 10% bovine serum albumin, 1% penicillin/streptomycin, and 3 mM β-glycerophosphate), additionally supplemented with calcium to a concentration of

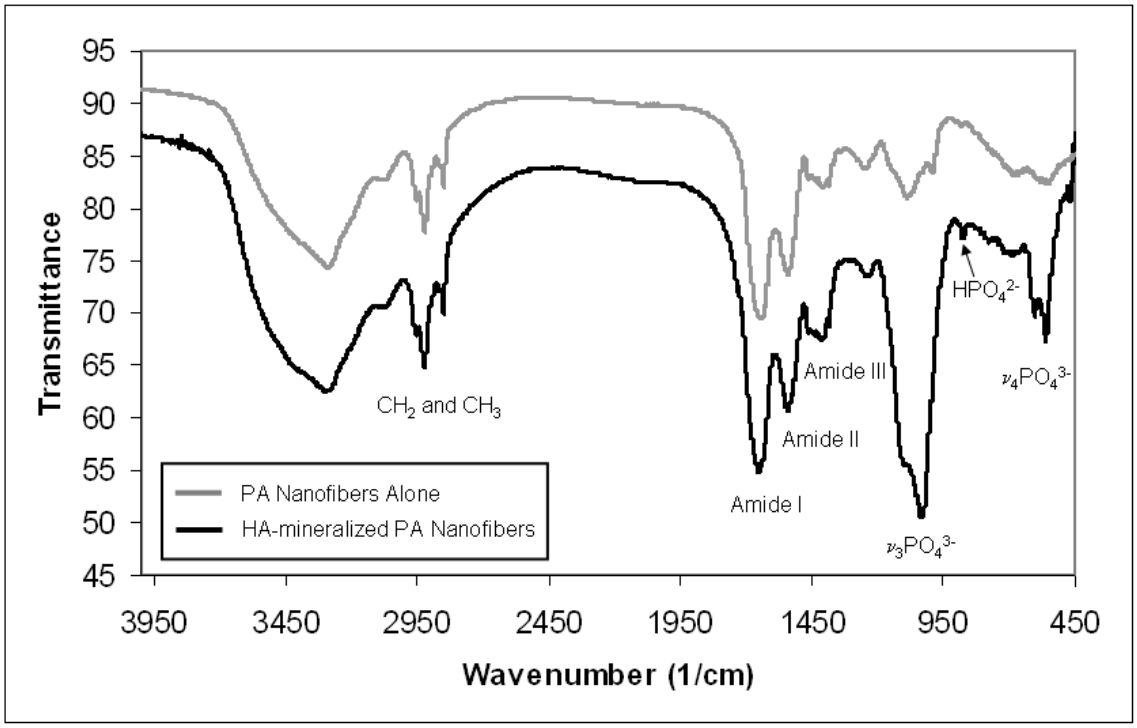
170 mM $CaCl_2$. All gels were then incubated at 37°C and 100% humidity in 5% CO_2 for 2 hours to insure complete gelation. Subsequently, 500 μL of culture medium (no calcium supplement) were added to chamber wells to cover the bubble of each gel. Samples were incubated as above, changing 250 μL of culture medium every 2 days.

Unmineralized gels were examined by wicking dilute suspensions of PA nanofibers from TEM grids. Grids were then negative stained by wicking an aqueous solution of 2.5% phosphotungstic acid from the grids and allowing to air dry. Gelled samples to be evaluated by electron microscopy were removed from their culture wells, fixed for 1 hour in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer. Fixed samples were then dehydrated in graded ethanol solutions (50%, 70%, 80%, 90%, 95%, 100%). Ethanol was exchanged in two changes of propylene oxide, followed by a 50% propylene oxide/EPON epoxy resin mix. Samples were infiltrated with resin by successive exchanges with pure EPON before final epoxy curing at 50°C for 24 hours, 60°C for 24 hours, 70°C for 24 hours. Sections were microtomed to 100 nm sections and placed onto copper TEM grids. Sections were viewed on a JEOL 100CX transmission electron microscope at 80kV. Gels to be examined by scanning electron microscopy were fixed and dehydrated as above, but were then critical point dried by CO_2 exchange. Dried gels were sputter-coated with 6 nm Au-Pd and examined in a Hitachi S4500 Scanning Electron Microscope at 5-10 kV.

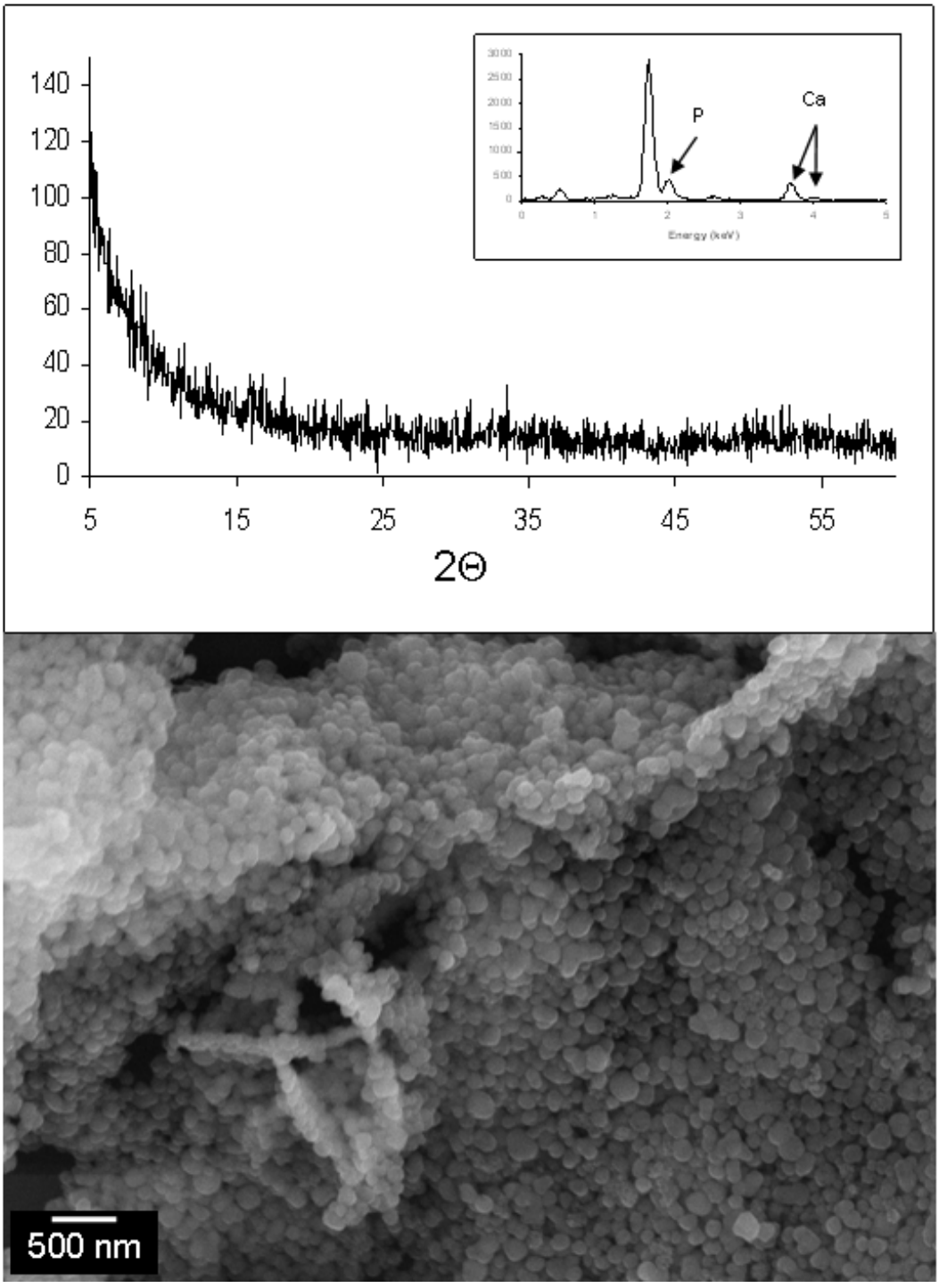
Both Transmission Fourier transform infrared spectroscopy (FTIR) and powder X-ray Diffraction (XRD) were performed on lyophilized powders of

mineralized and non-mineralized gels. For FTIR the powders were mixed with potassium bromide and pressed into pellets for analysis using a Bio-Rad FTS-40 FTIR spectrophotometer. XRD studies were performed by dispersing the lyophilized PA on a “zero-background” quartz holder for examination with a Siemens D500 theta/2-theta diffractometer using Cu-Ka radiation at 40kV and 30mA.

To determine if ALP could enzymatically liberate phosphates from the PA, samples of both assembled and non-assembled PA were probed using a commercial Malachite Green Assay (BioAssay Systems, Hayward, CA.) Free phosphates produced were determined by the absorbance measured at 650 nm on a plate-reader. The PA was tested at a concentration of 10 mg/mL in pure water (or the aqueous calcium chloride solution used to gel the PA), and was treated with 0.5 units of alkaline phosphatase for 10 minutes before measurement.



Supplemental Figure 1: Fourier Transform Infrared Spectra of the self-assembled PA nanofibers and PA nanofibers mineralized with hydroxyapatite (HA).



Supplemental Figure 2: Amorphous calcium phosphate formed when β-glycerophosphate was replaced by sodium phosphate (Na_2HPO_4) as the phosphate source in the mineralization solution. X-ray diffraction and energy dispersive x-ray spectroscopy confirm that the aggregated spherical materials visible in the scanning electron micrograph are amorphous calcium phosphate.