Supporting Information

Fang et al. 10.1073/pnas.1106228108

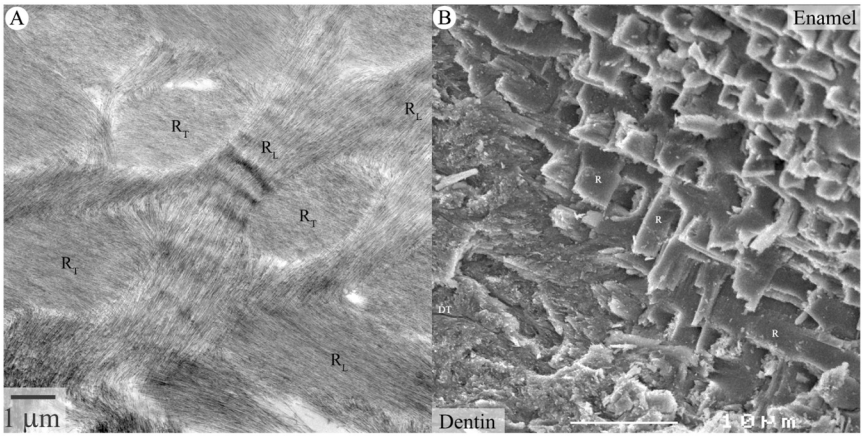


Fig. S1. Intricate three-dimensional structure comprised of interwoven arrays of elongated calcium phosphate crystals—enamel rods in (*A*) transmission electron microscopy micrographs of unstained resin embedded sections of forming murine enamel. R_T, transversely sectioned rod; R_L, longitudinally sectioned rod. (*B*) Scanning electron micrograph of a fractured erupted murine incisor. R, rods.

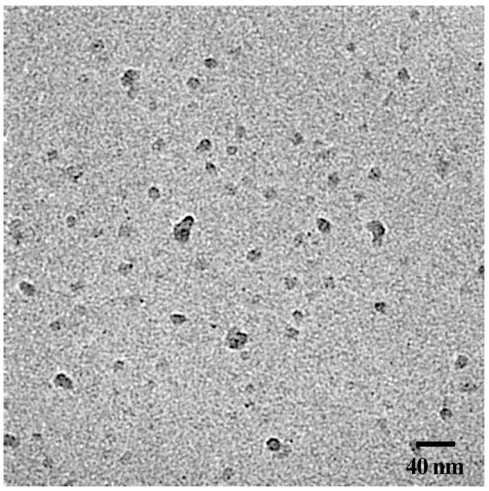


Fig. S2. Cryoelectron micrograph of rM179 at pH 8.0, 5 min after the beginning of the experiment.

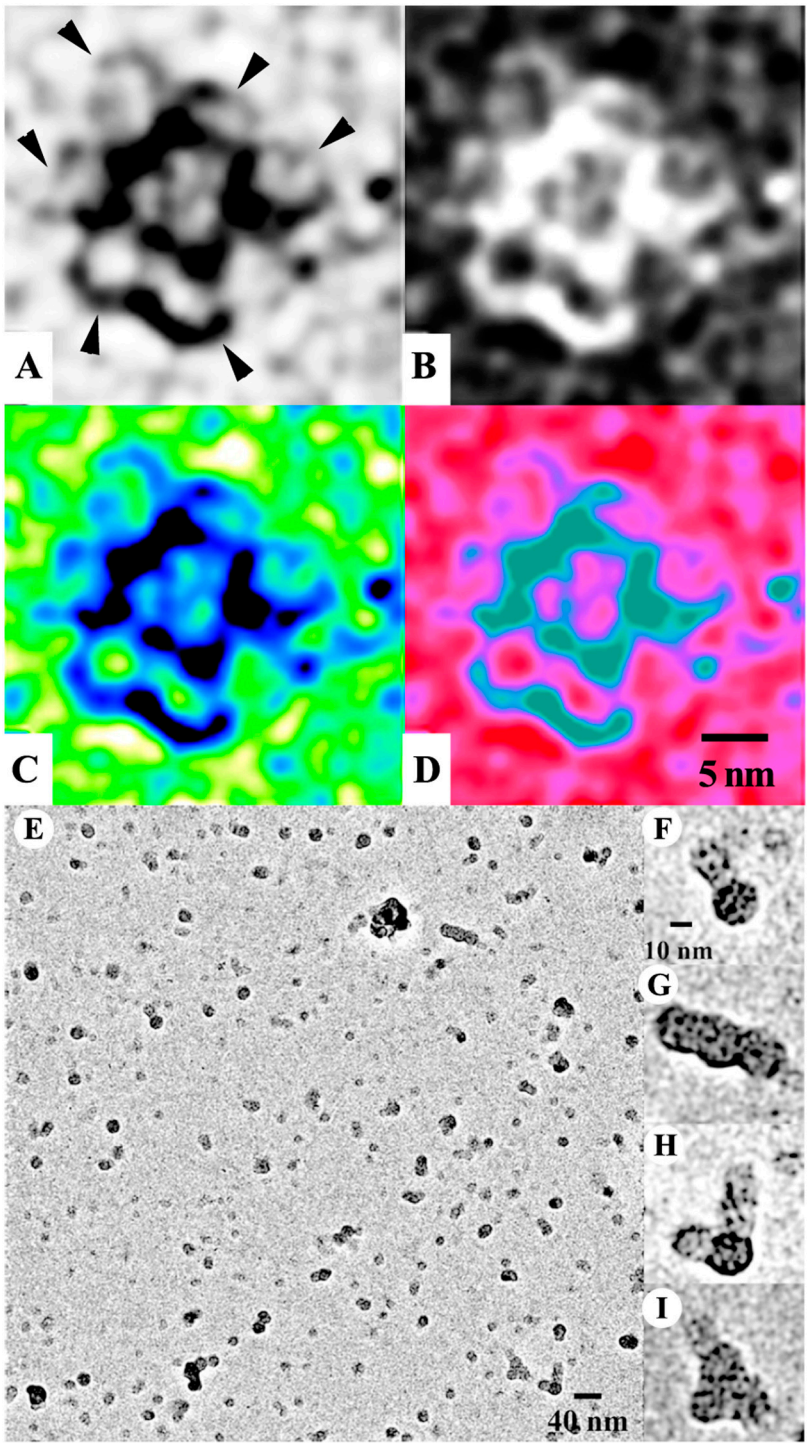


Fig. S3. (*A*–*D*) Larger views of a nanosphere three from Fig. 1*H* in (*A*) gray scale positive, (*B*) gray scale negative, inverted fire (*C*), and ice (*D*) look-up tables created using ImageJ image processing software. Arrowheads indicate individual oligomers comprising the nanosphere. (*E*) Cryoelectron micrograph of rM179 at pH 8.0, 10 min after the beginning of the experiment. Several nanosphere chains are present in the field of view. (*F*–*I*) Close-ups of the nanosphere chains from *E*.

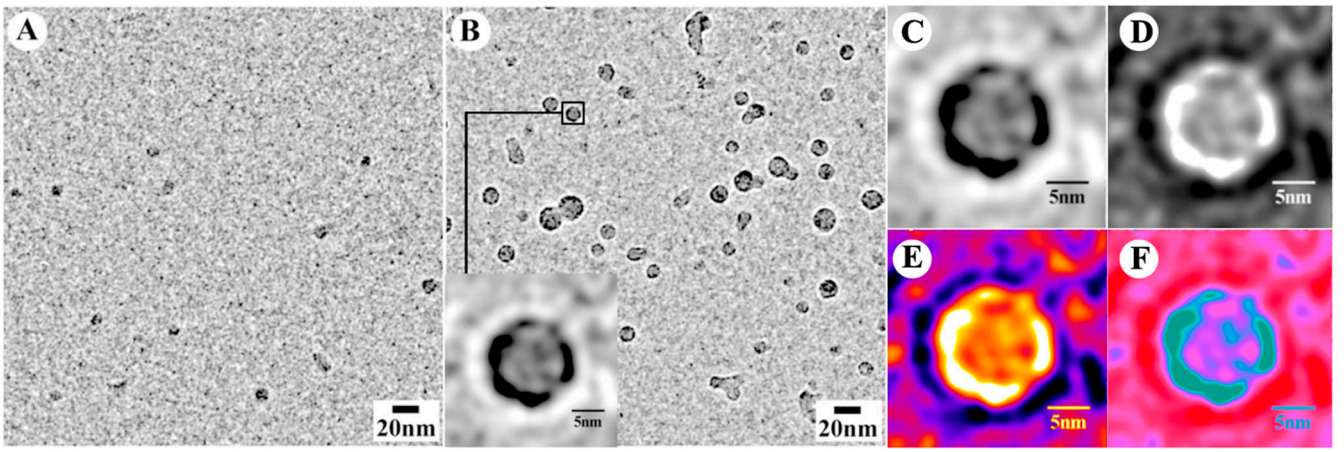


Fig. S4. Cryoelectron micrographs of rM166 after 1 (*A*) and 10 min (*B*) in PBS at pH 8.0. The inset in Fig. 4*B* shows a single nanosphere at higher magnification. (*C*–*F*) Larger views of a nanosphere in the inset; (*C*) gray scale positive, (*D*) gray scale negative, inverted fire (*E*), and ice (*F*) look-up tables created using ImageJ image processing software. Note that, although there are some small variations of density present in the nanosphere core reflecting a certain degree of electron density variations, they are not as strong or structurally defined as the ring-like structures comprising the rM179 nanospheres presented in Fig. 1 and Fig. S3.

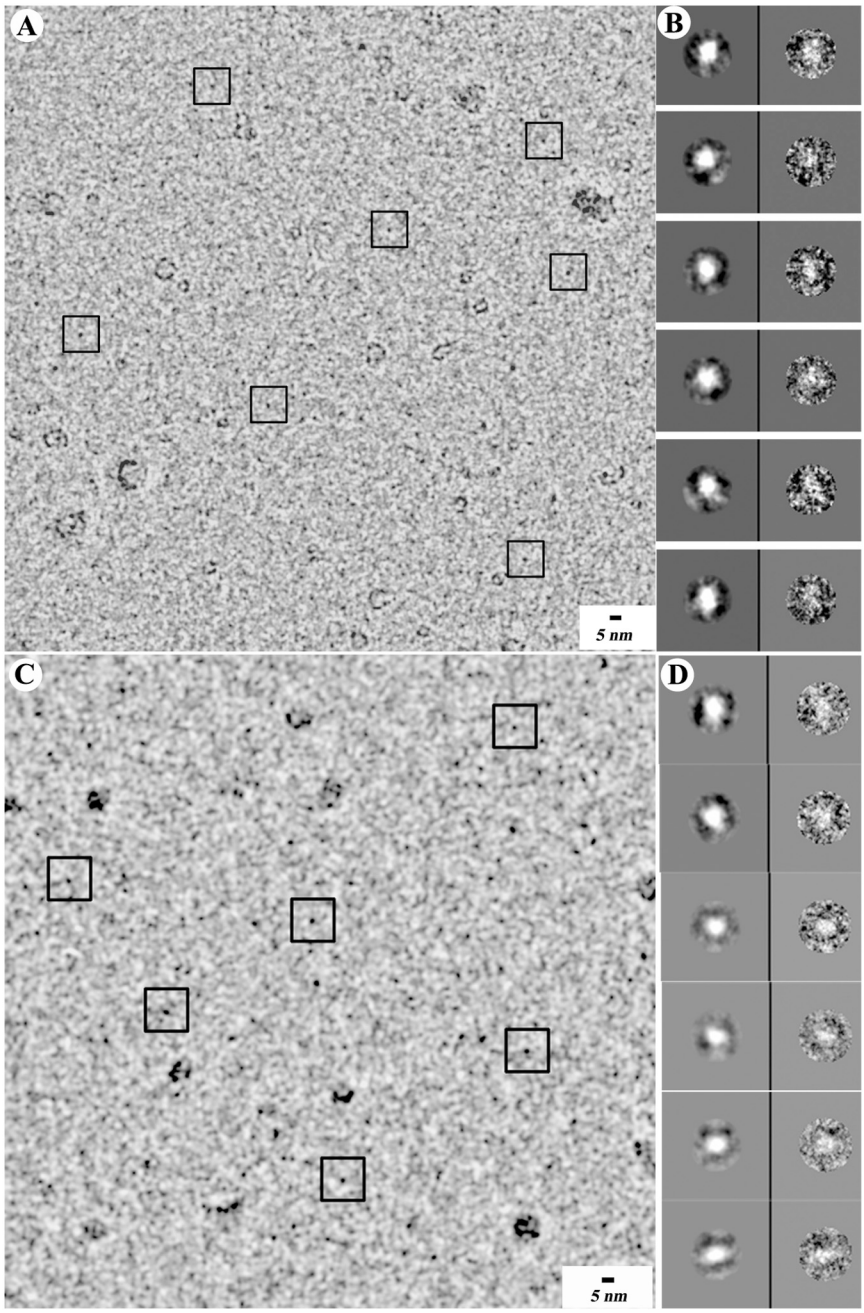


Fig. S5. Single-particle reconstruction of rM179 monomers. (*A*) A representative cryoelectron micrograph of amelogenin rM179 in PBS at pH 8.0 after 1 min with boxed monomer particles. (*B*) Comparison of computer-generated model projections of the rM179 particles (*Left*) with class average of individual particles (*Right*). (*C*) A representative cryoelectron micrograph of amelogenin rM166 in PBS at pH 8.0 after 1 min with boxed monomer particles. (*D*) Comparison of computer-generated model projections of the rM166 particles (*Left*) with class average of individual particles (*Right*).

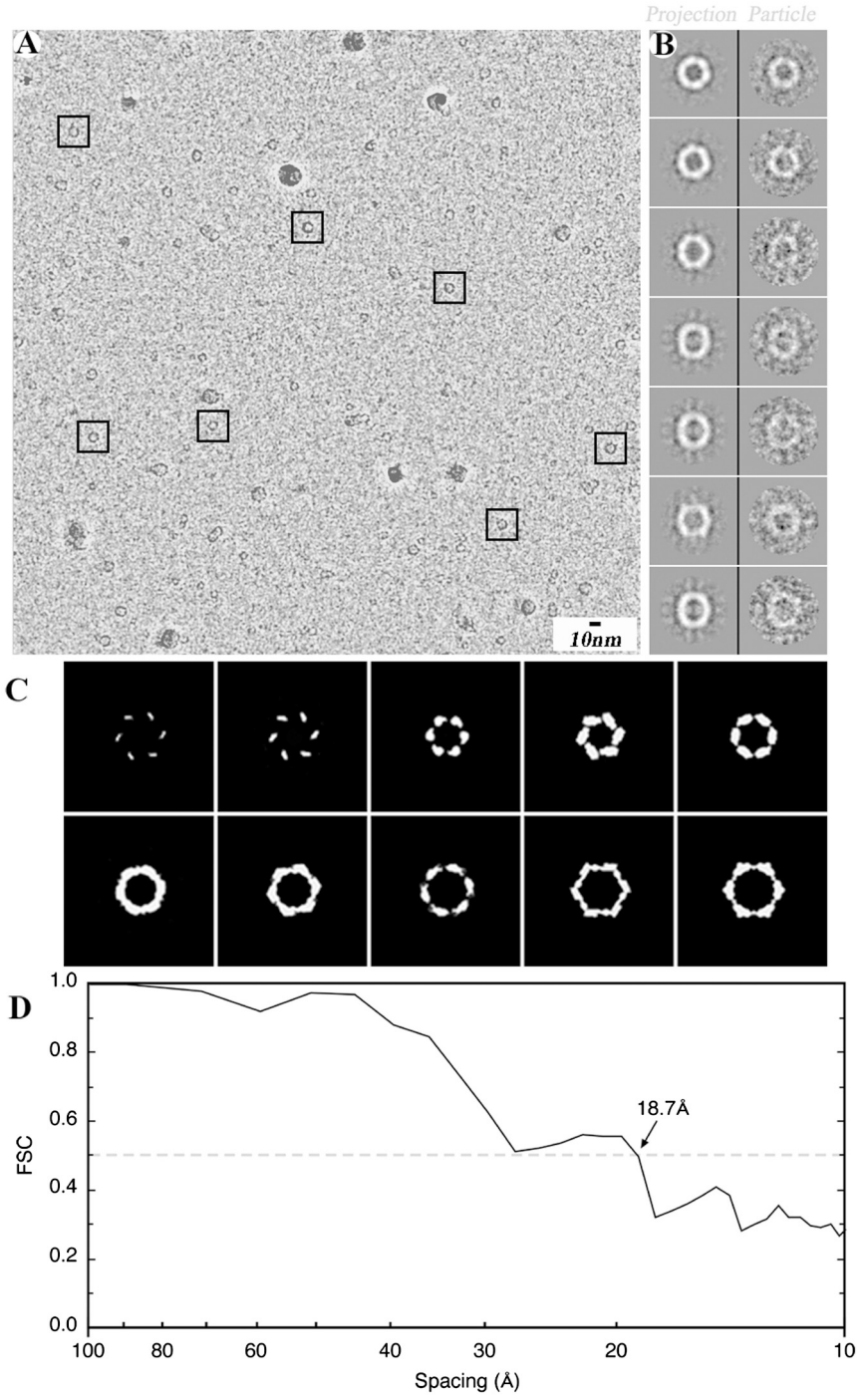


Fig. S6. *A*) A representative cryoelectron micrograph of amelogenin rM179 in PBS at pH 8.0 after 1 min with boxed oligomer particles. *B*) Comparison of computer-generated model projections (*Left*) with class averages of individual particles. (*C*) Cross-section view of density map of rM179 dodecamer along *z* axis from top to middle with the spacing between the sections of 10.7 Å. (*D*) The map of resolution of rM179 dodecamer was determined to be at 18.7 Å using the Fourier shell correlation (FSC) (0.5 threshold criteria) of two 3D maps independently built from half datasets.

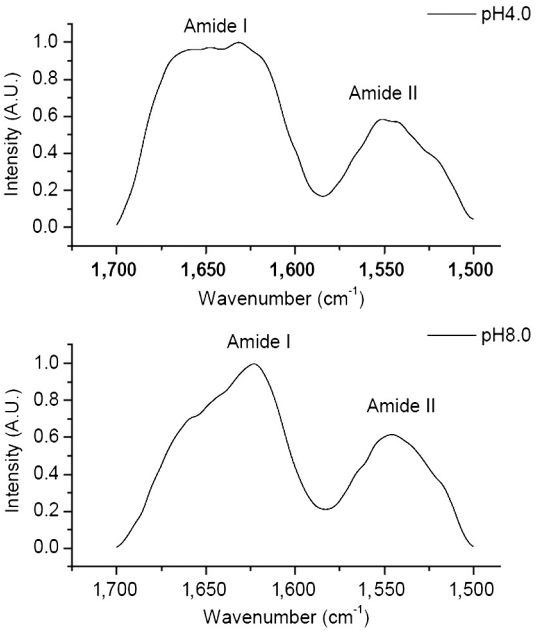


Fig. S7. FTIR spectra of rP172 in aqueous solution at pH 4.0 and in PBS at pH 8.0. Note significant changes in the Amide I band profile. Specifically, while at pH 4.0, the spectrum features a broad Amide I band with several maxima, the Amide I band at pH 8.0 features a sharp and strong maximum at 1,624 cm^{-1}, indicating that a large proportion of the protein exists in β-sheet conformation. FTIR spectroscopy and data analysis were conducted as previously described (1).

1 Elangovan S, Margolis HC, Oppenheim FG, Beniash E (2007) Conformational changes in salivary proline-rich protein 1 upon adsorption to calcium phosphate crystals. *Langmuir* 23:11200–11205.

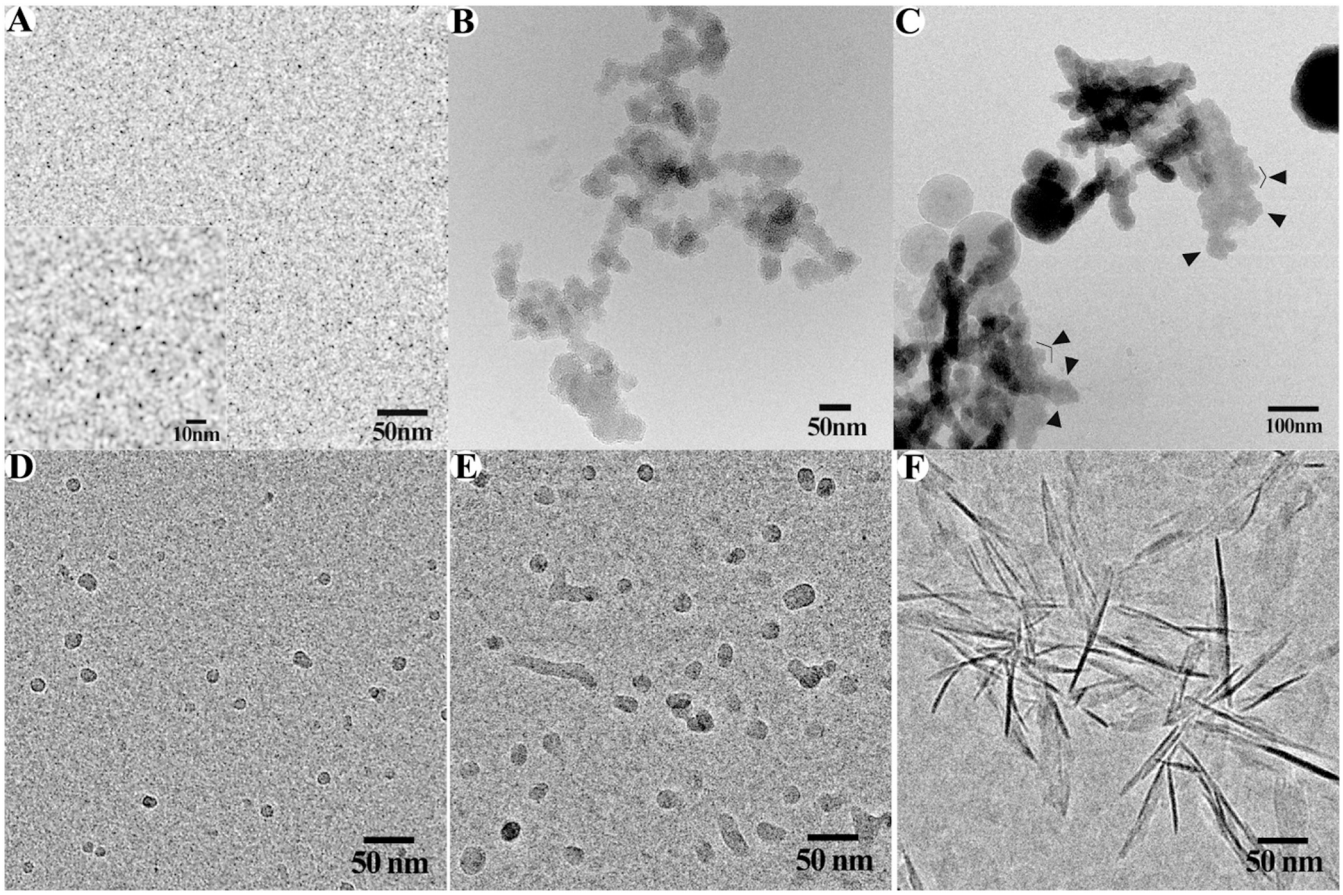


Fig. S8. Cryoelectron microghraphs of calcium phosphate mineralization in the absence of the proteins (*A*–*C*) and with rM166 (*D*–*F*). Without proteins: (*A*) 10 min in the reaction; inset contains an area from the same field of view at higher magnification. (*B*) Chains of spherical mineral aggregates at 30 min. (*C*) After 2 h, plate-shaped mineral particles are abundant. Note the straight crystalline faces with approximately 120° angles between them (arrowheads) suggesting the crystalline nature of the mineral. With rM166: (*D*) After 10 min in the reaction, in addition to the individual prenucleation clusters, numerous spherical aggregates, approximately 20 nm in diameter form; we have interpreted these as aggregate complexes of rM166 protein and prenucleation clusters. (*E*) At 30 min, the reaction product looks very similar to the 10-min sample, except some of the aggregates start to fuse. (*F*) Finally, after 120 min, randomly oriented ribbon-like mineral particles appear.

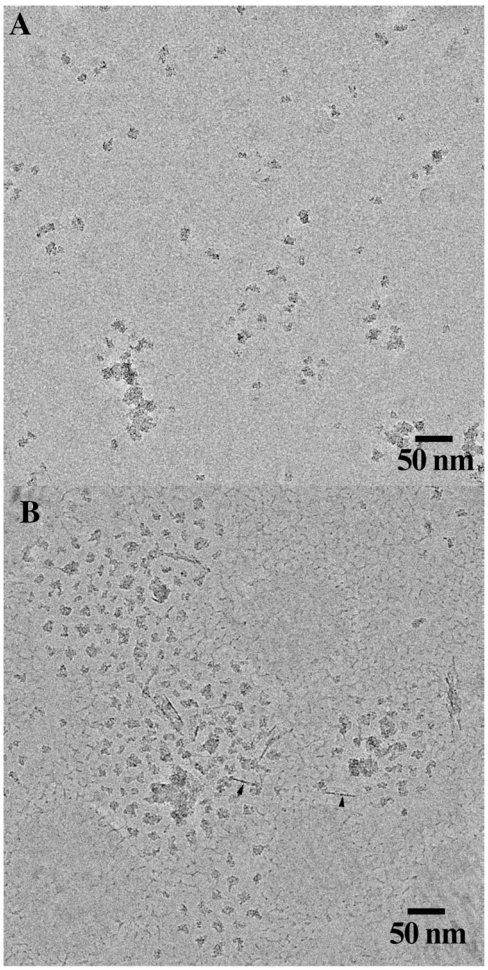


Fig. S9. Cryoelectron micrographs of calcium phosphate mineralization in the presence of full-length amelogenin rM179. (*A*) Ten minutes in the reaction (enlargement of Fig. 3*A*); (*B*) Thirty minutes in the reaction (enlargement of Fig. 3*B*); arrowheads point toward the linear chains of the prenucleation clusters.

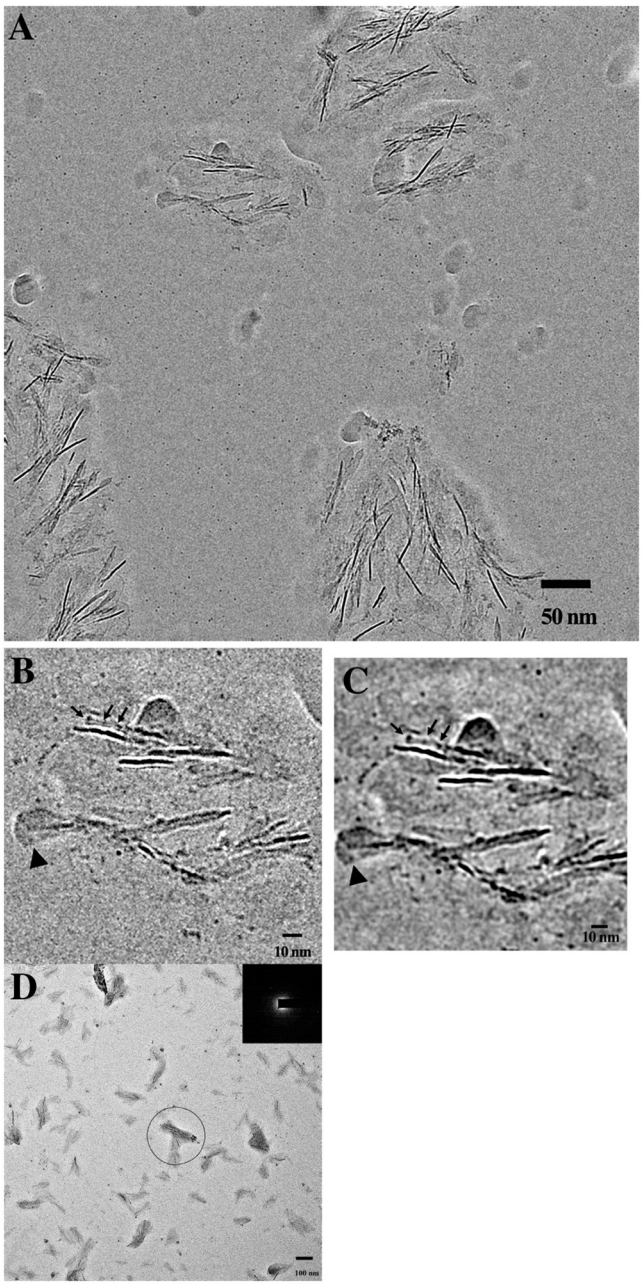
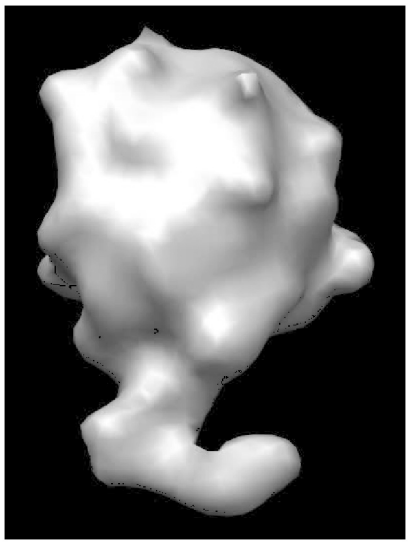
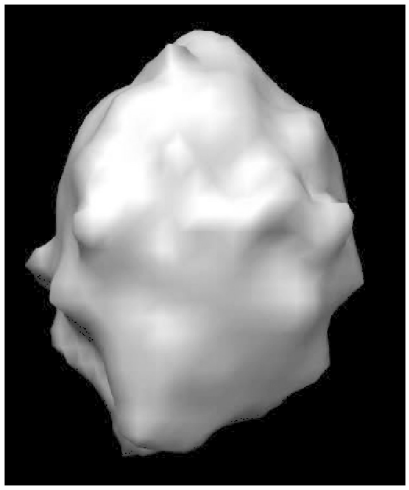


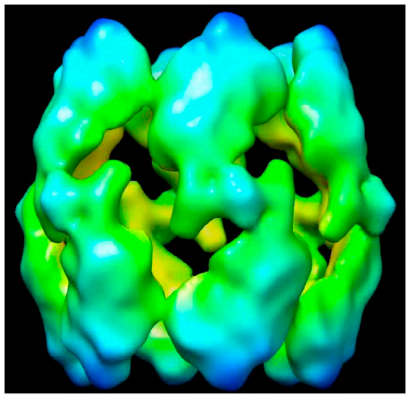
Fig. S10. Cryoelectron micrographs of calcium phosphate mineralization in the presence of full-length amelogenin rM179 120 min in the reaction. (*A*) Larger image of Fig. 3*C*; (*B*) larger image of the area presented in Fig. 3*I*; (*C*) the same image after 3.5-nm low-pass filtering. Arrows point at the prenucleation clusters aligned with an elongated mineral particle. Note the strands connecting these particles, likely made of amelogenin protein. Large arrowhead points to an envelop of organic material surrounding a tip of an elongated mineral particle. (*D*) Transmission electron micrograph of freeze-dried sample of mineral formed in the presence of rM179, 2 h after the beginning of the reaction. Based on the electron diffraction analysis (*Inset*), the mineral is not crystalline at this point. The circle outlines the mineral bundle from which the diffraction was taken.



Movie S1. Single-particle reconstruction model of rM179 monomer.
Movie S1 (MPG)



Movie S2. Single-particle reconstruction model of rM166 monomer.
Movie S2 (MPG)



Movie S3. Single-particle reconstruction model of rM179 dodecamer.
Movie S3 (MPG)