

## RESEARCH REPORTS

### Biological

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## APPENDIX

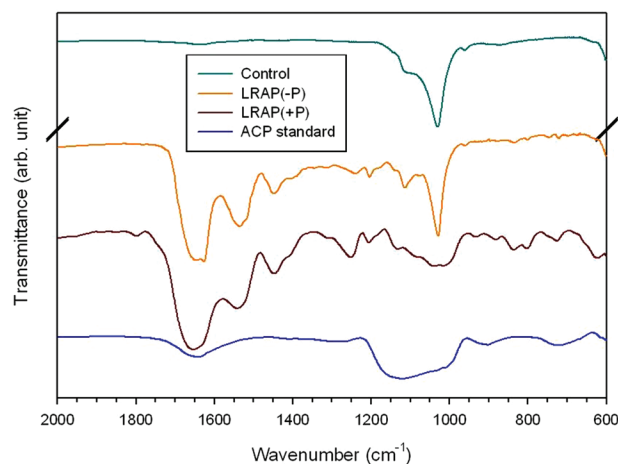
### Dynamic Light-scattering (DLS)

Dynamic light-scattering (DLS), as used in the present study, provides an indirect measure of “particle” size based on particle diffusion (mobility) characteristics that are, in part, related to mass. The size (hydrodynamic radius,  $R_H$ ) of LRAP particles under noted experimental conditions was determined, as previously described (Wiedemann-Bidlack *et al.*, 2007). Larger particles or interconnected assemblies of nanoparticles will diffuse more slowly than smaller or isolated nanoparticles, resulting in larger  $R_H$  values or off-scale scattering. In the present study, DLS was used to obtain information on the onset of changes in LRAP aggregation (as reflected by changes in  $R_H$  values), as a function of solution pH and/or the addition of calcium. Since DLS alone does not provide information on the structure of aggregates formed, parallel studies were carried out using transmission electron microscopy (TEM), as described in the main text. Although TEM findings, in general, reflect changes in the extent of aggregation as seen by DLS, TEM also provides information on how the various LRAPs assemble. Observed differences in these assemblies are seen through an examination of representative TEM images presented in the manuscript.

### Fourier Transform-Infrared (FT-IR) Spectroscopy Analyses

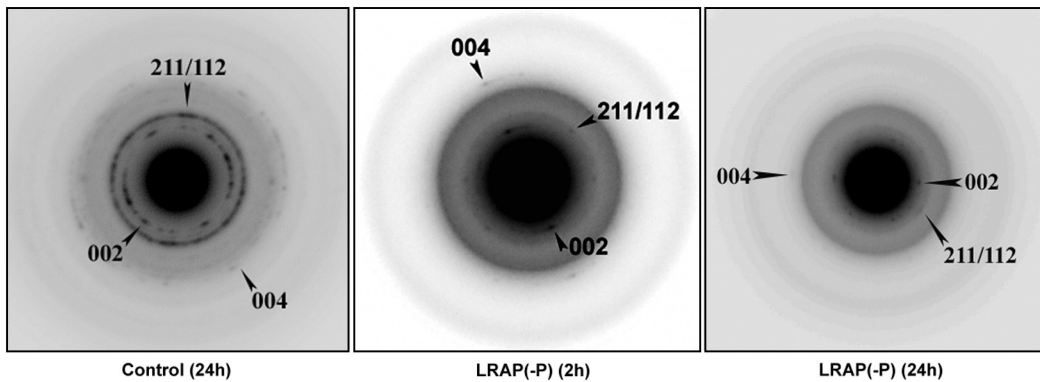
For comparison, an FT-IR spectrum for ACP alone is also presented (Appendix Fig. 1) using pyrophosphate-stabilized ACP, prepared by a modification of a previously described approach (Beniash *et al.*, 2009). Pyrophosphate-stabilized ACP was prepared by the mixing of 100 mM  $\text{CaCl}_2$  and 100 mM  $\text{Na}_2\text{PO}_4$  with  $\text{Na}_4\text{P}_2\text{O}_7$  (final concentration: 1.2  $\mu\text{M}$ ) in distilled water to

## Leucine-rich Amelogenin Peptides Regulate Mineralization *in vitro*

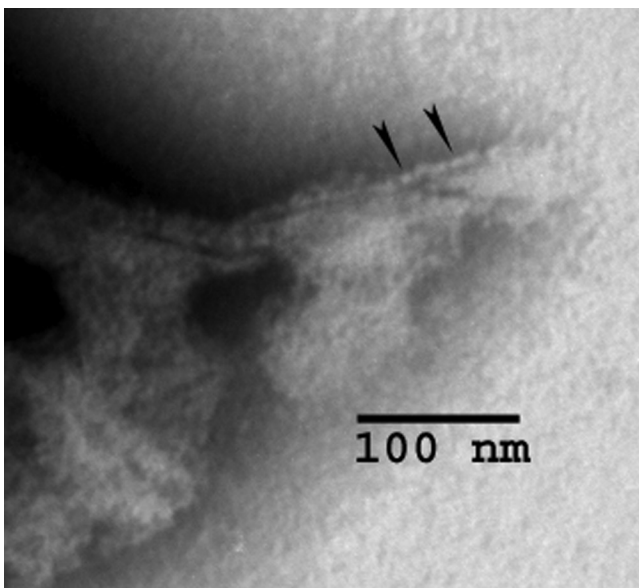


**Appendix Figure 1.** FT-IR analyses of the mineral phases found at 24 hrs after mineralization experiments were begun in the absence (control) and presence of LRAP(+P) and LRAP(-P). As found by SAED (Fig. 4, main paper), FT-IR spectra obtained for control samples and products obtained in the presence of LRAP(-P) were consistent with the formation of hydroxyapatite ( $\nu_3$  of  $\text{PO}_4^{3-}$ : 1102, 1035  $\text{cm}^{-1}$ ), while spectra of samples obtained in the presence of LRAP(+P) were consistent with amorphous calcium phosphate (ACP). The amide I (1653  $\text{cm}^{-1}$ ), II (1553  $\text{cm}^{-1}$ ), and III (1400  $\text{cm}^{-1}$ ) peaks consistent with protein were observed in the presence of LRAP(+P) and LRAP(-P).

final concentrations of 5 mM and 3 mM, respectively, under ambient conditions with stirring. The resulting pH is  $\sim 7.1$ . Thirty min after the beginning of the reaction, the mineral suspension was centrifuged at 8000  $\times g$  at 20°C for 5 min. The precipitate was then lyophilized at -20°C.



**Appendix Figure 2.** Magnification of SAED patterns of control and LRAP(-P) samples obtained from mineralization experiments at 2 hrs and 24 hrs. In the presence of LRAP(-P), narrow arcs of the 002 and 004 reflections could be observed, indicating an alignment of hydroxyapatite crystals in the c-axis direction; whereas, in the control, the 002 and 004 reflections appeared as rings, consistent with randomly oriented hydroxyapatite crystals.



**Appendix Figure 3.** TEM images of negatively stained (PTA) mineralization samples at 24 hrs in the presence of LRAP(-P), showing an alignment of the LRAP(-P) nanospheres along the edges of forming crystals (arrows).

## APPENDIX REFERENCES

- Beniash E, Metzler RA, Lam RS, Gilbert PU (2009). Transient amorphous calcium phosphate in forming enamel. *J Struct Biol* 166:133-143.
- Wiedemann-Bidlack FB, Beniash E, Yamakoshi Y, Simmer JP, Margolis HC (2007). pH triggered self-assembly of native and recombinant amelogenins under physiological pH and temperature *in vitro*. *J Struct Biol* 160:57-69.