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Exon4 Amelogenin Transcripts in Enamel Biomineralization

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Appendix

Comparison of Predicted Calcium and Phosphate Binding Sites in Amelogenin Isoforms

We used a bioinformatics approach previously published by Horst and co-workers (Horst et al. 2010) to investigate the possibility that, in addition to increased binding to hydroxyapatite (HAP), amelogenins derived from transcripts containing exon4 (AMG+4) may have increased binding to calcium and phosphate. This approach calculates the likelihood of interaction for each amino acid residue by building logistic regressions trained to maximize scores for: 591 residue side-chains non-covalently bound to phosphate ions among 39,641 residues in crystal diffraction structures of 190 35% sequence-non-redundant proteins in the Protein Databank (Berman et al. 2000; Horst and Samudrala 2010; Martinez-Avila et al. 2012); or 696 calcium-binding residues among 72,355 in 294 protein structures (Horst and Samudrala 2010). The model was developed with known amino acid sequences in crystallized protein structures with known calcium and phosphate binding, with the intent that this could then be used to predict these interactions with amino acid sequences of proteins that had not been crystallized, such as amelogenin.

Here, we applied these methods to predict functional interactions for M189 (AMG-4) M194 (AMG+4), and found that exon4-translated peptide increased the predicted interactions with calcium and phosphate (Appendix Fig.). It should be noted that we considered serine-16 as not phosphorylated, which is thought to be the state of roughly half the amelogenin population. The reason for this is that there are not enough well-characterized interactions of phosphoserines to build and apply sequence-analytic predictive methods. However, we do acknowledge that binding to calcium is known to increase in amelogenin fragments when serine 16 is phosphorylated (Beniash et al. 2009; Kwak et al. 2009).

This bioinformatics approach suggested that exon4-translated peptide may directly bind calcium and phosphate, and suggests the possibility that this peptide sequence may therefore also stabilize HAP precursors, including amorphous calcium phosphate (ACP). A stabilization of ACP at the transition stage of enamel formation could provide a reservoir of calcium and phosphate to allow for the rapid mineral formation into the porous enamel structure that is left after the rapid removal of matrix proteins that occurs at the transition between secretory and maturation-stage enamel formation. Further studies are required to determine the interaction between AMG+4 and HAP or its precursors in enamel biomineralization.

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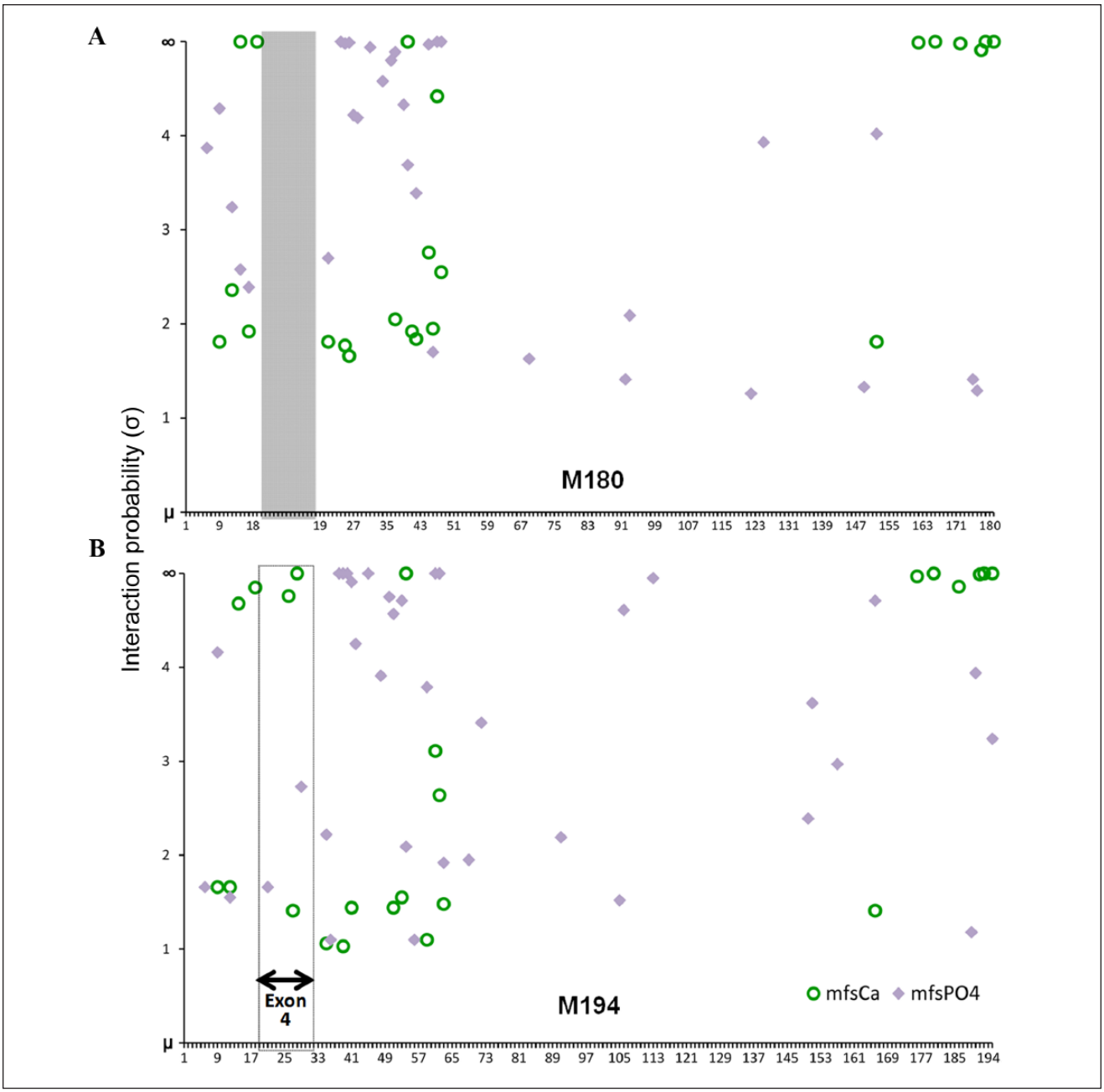

Appendix Figure. Predicted binding of amelogenin to calcium and phosphate. The amelogenin region encoded by exon4 is predicted to interact directly with calcium and phosphate. The likelihood of interaction for each amino acid residue with calcium (○) and phosphate (♦) was calculated for (**A**) M194 with exon4-translated peptide, or (**B**) without exon4. Interaction likelihood values are shown for each residue with significant predictions. The greater the number, the higher the likelihood of predicted interactions.

Appendix References

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