

## Effects of a common buffer on calcium phosphate nucleation: Does TRIS modify the association of calcium and phosphate ions?

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It is well known that organic molecules can have a dramatic effect on the nucleation and growth of biominerals. Nonetheless, experiments using tris(hydroxymethyl) aminomethane (TRIS) to control the pH, often do not consider its potential impact on the reaction. Through both experiments and molecular dynamics simulations, we show that TRIS inhibits the nucleation of calcium phosphate by initially interacting with the calcium and phosphate ions in solution and, in subsequent steps, with the aggregates formed by those ions. The presence of TRIS increases the equilibrium Ca-P coordination number towards values comparable to those seen in the units previously proposed by Habraken *et al* [1] to explain their experiments. Simulations performed in the absence of TRIS, show a coordination of three only when the clusters start to densify; the average is always lower than for simulations where TRIS is present. In these cases, clusters form with a higher ratio of phosphate to calcium but cannot densify until enough calcium diffused to the cluster and equilibrates the charge. This can explain the inhibitory effect of TRIS and the formation of dense liquid phases induced by the presence of charged polymers as proposed in the PILP model of Gower [2].

[1] W.J.E.M Habraken, J. Tao, L.J. Brylka, H. Friedrich, L. Bertinetti, A.S. Schenk, A. Verch, P.H.H. Bormans, P.M. Frederik, J. Laven, P. van der Schoot, B. Achmayer, G. de With, J.J. deYoreo, N.A.J.M. Sommerdijk. *Nat. Comms* 4, (2013) 1507. [2] L.B. Gower and D.J. Odom, *J. Cryst. Growth* 210 (2000), 719.