# THE ROLE OF ALKALINE PHOSPHATASE IN PHYSIOLOGICAL AND PATHOLOGICAL CALCIFICATION - NOVEL THERAPEUTIC STRATEGIES

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Calcification is normally restricted to skeletal tissues due to the co-expression in bone and cartilage of tissue-nonspecific alkaline phosphatase (TNAP) and fibrillar collagens. TNAP's primary function is to degrade extracellular inorganic pyrophosphate (*e*$PP_i$), a potent mineralization inhibitor, produced ectoplasmically by the enzymatic activity of NPP1 and transported to the extracellular milieu by ANK. Mice deficient in TNAP (*Akp2*$^{-/-}$) develop hypophosphatasia (HPP) characterized by greatly elevated levels of *e*$PP_i$, which lead to rickets and osteomalacia. Consequently, natural substrates for this ectoenzyme accumulate extracellulary including inorganic pyrophosphate ($PP_i$), an inhibitor of mineralization, and pyridoxal 5'-phosphate (PLP), a cofactor form of vitamin $B_6$. Babies with the infantile form of HPP often die with severe rickets, hypercalcemia and vitamin $B_6$-dependent seizures. There is no established treatment. We bio-engineered human TNAP with a C-terminus extended by the Fc region of human IgG for one-step purification, and a deca-aspartate sequence ($D_{10}$) for targeting to mineralizing tissue (sALP-Fc$D_{10}$). *Akp2*$^{-/-}$ mice receiving high-dose sALP-Fc$D_{10}$ grew normally and appeared well without skeletal or dental disease or epilepsy. Clinical trials in patients with infantile HPP are currently ongoing. In turn, low levels of extracellular inorganic pyrophosphate (e$PP_i$) lead to soft-tissue calcification abnormalities, including vascular calcification. Using cultures of vascular smooth muscle cells (VSMC), we have established that VSMCs from *Enpp1*$^{-/-}$ and *ank/ank* mutant mice upregulate TNAP expression and calcify more than control cells. We have also found that TNAP is upregulated in the aortas of uremic rats. We are currently involved in a large scale Medicinal Chemistry effort aimed at testing the hypothesis that inhibition of TNAP's pyrophosphatase activity will prevent/reverse medial calcification *in vivo*.