# Characterizing Novel Methylation and Cleavage Pathways in Borosin Natural Product Precursor SamA1

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Borosins are a family of RiPPs that are characterized by post-translational ?-N-methylations on their peptide sequence. Since ?-N-methylations, though desirable, add difficult synthesis and purification steps that result in loss of product, the borosin family provides the compelling possibility of discovering already-methylated, stable, bioactive, and orally bioavailable drugs. I investigated the post-translational modifications of borosin precursor SamA1. I successfully optimized the heterologous expression of the wildtype CVFF-SamA1, a cleavage site mutant, CVFP-SamA1, and a mutant lacking its putative core domain, NoCore-SamA. SamA1 and its mutants were incubated with SamP and analyzed through gel electrophoresis to identify the presence of a cleaved product. As expected, no cleavage occurred with unmethylated SamA1, while methylated SamA1 was only cleaved in the presence of SamP. However, without the presence of SamP, there still appeared to be a cleaved product for both unmethylated and methylated SAMA1 protein with the native cleavage site (CVFF-1 and CVFF-2, respectively) run through Size Exclusion Chromatography (SEC). Additionally, even with an inactive mutant methyltransferase co-cultured with SamA1, cleavage still occurred in the presence of SamP. In a SamA1 protein with a mutated cleavage site (CVFP), there still appeared to be cleaved products with and without SamP. These results may indicate that SamP may recognize not only a methyl domain, but also the presence of a residual methyltransferase enzyme, however it will be necessary to repeat the in vitro assay to determine whether this represents a contamination artifact or a novel function.

**Awards Won:**