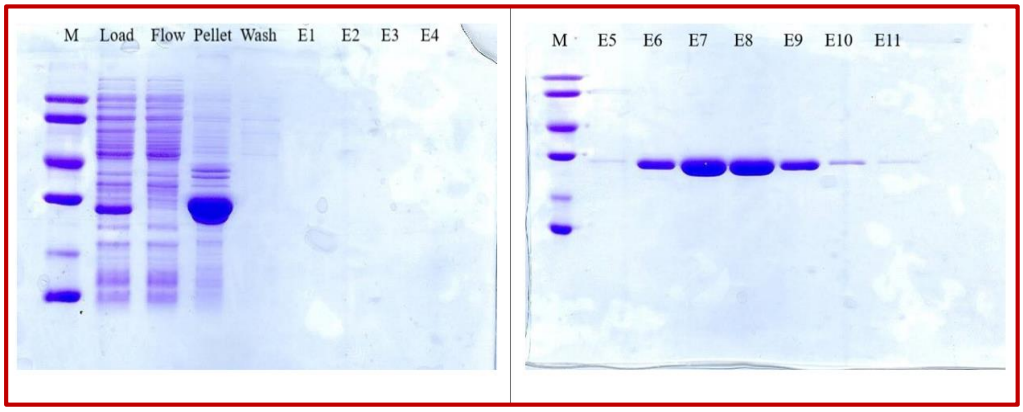
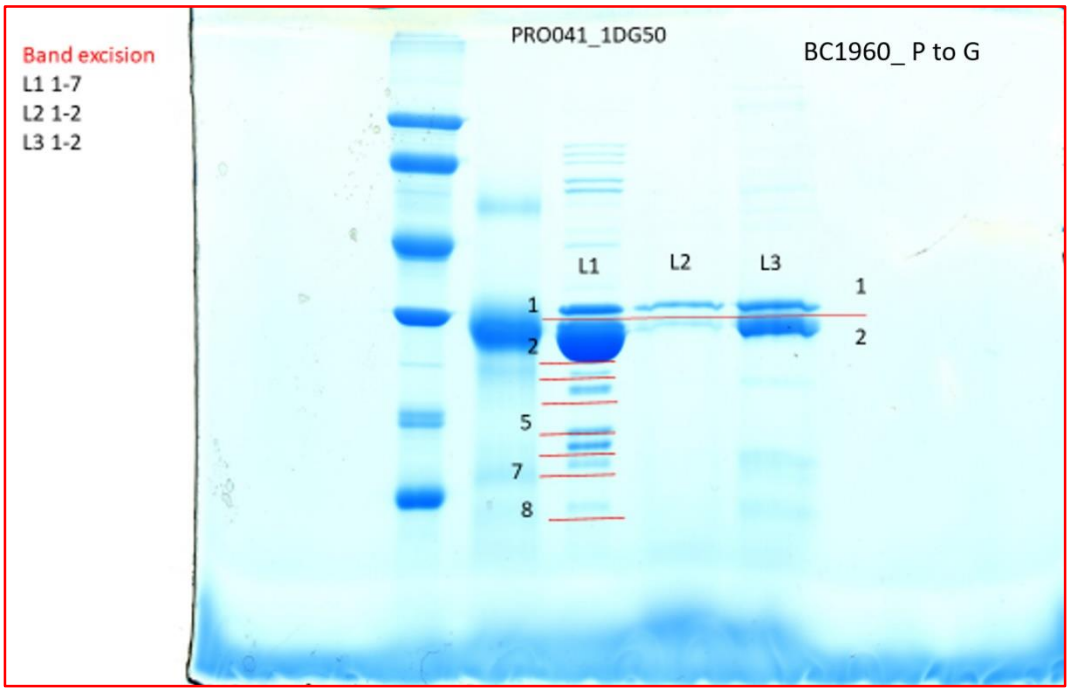
Implementation, achievements and opportunities

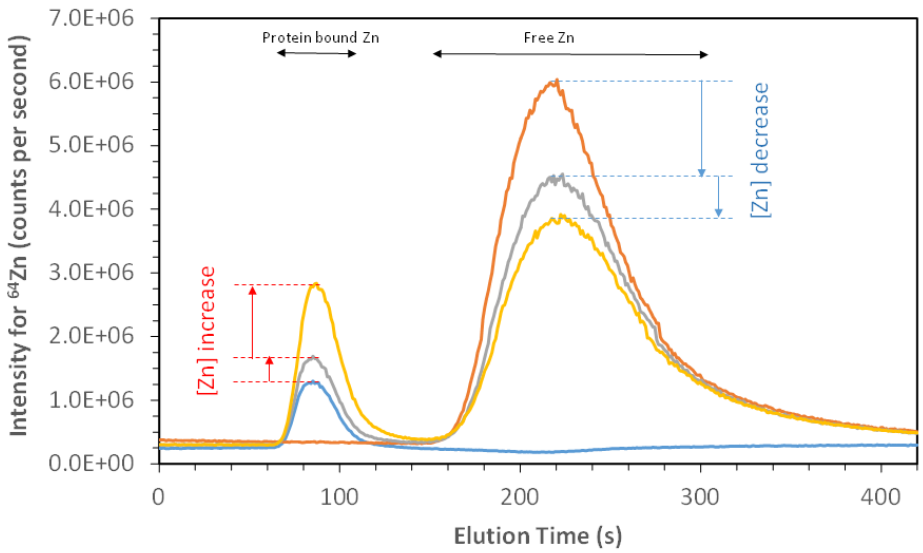
WP1, WP2



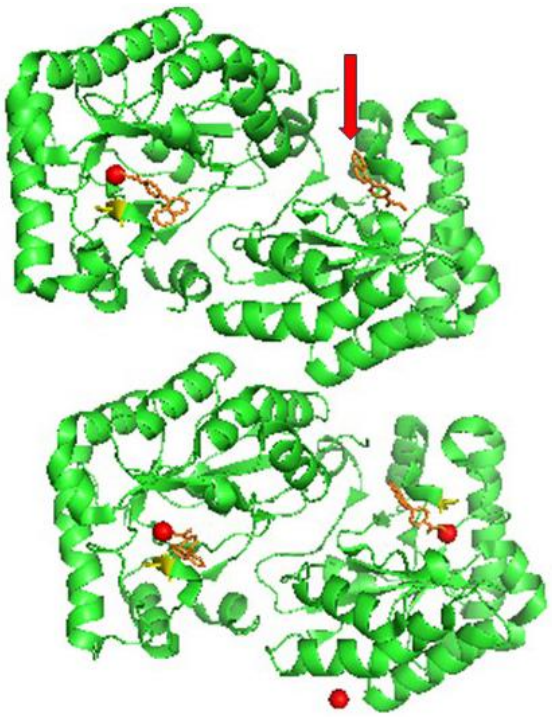
Purification of the BC1960 mutant R169A which was produced to ***explore the environment of the self-hydroxylation centre*** *P171. Reversing such mutations with suitable amino acid substitutions plays a key role in the reactivation of inactive PDAs (such as the pseudoenzyme Ba3943). 5-10 mg of more than 90% pure protein were obtained from each purification run.*



The Bc1960 P171G mutant displays pronounced degradation which starts from the N-terminus and manifests itself in form of a pattern of multiple bands. Samples L1-L3 were obtained using different purification protocols dictated by the aggregation propensity of the protein, with L1 produced via native purification, and L2, L3 by purification after refolding. Red lines show the pattern of band excisions prior to subsequent mass-spec analysis ***which revealed an unusual hydroxylation pattern of Gly residues.***



ICP-MS analysis of ***Zn^{2+} bound to Bc1960***



Crystal structure of a complex of Bc1960 with a hydroxamic acid derivative. The loose Zn^{2+} binding in the active site manifests itself in form of ***variable metal bindings geometries or in the absence of metal****. A* ***non-uniform Cα hydroxylation pattern*** *of Pro is also established.*