

## Implementation, achievements and opportunities

### WP5

#### Links between active site $Zn^{2+}$ and $C_{\alpha}$ hydroxylation

- In Bc1960 the active site  $Zn^{2+}$  is loosely bound in the hydroxylation/ catalytic site, as revealed by crystallographic and ICP-MS experiments. The Pro hydroxylation levels of Bc1960 are despite this very high, they are affected by molecular oxygen or by some hydroxylation inhibitors.
- Other PDAs however (e.g. Ba0330/ pdb id: 4V33; Bc1974/ pdb id: 5N1J; 5N1P) with lower hydroxylation levels compared to Bc1960, display fully occupied  $Zn^{2+}$  sites, suggesting a tighter interaction with the metals.
- Ba3941 and its mutants show no  $Zn^{2+}$  binding. The restored (in terms of hydroxylation and catalysis) mutant Ba3943 N94D V95D A183R ((PDB id 6HM9) displays some levels of active site  $Zn^{2+}$ .
- Overall,  $Zn^{2+}$  ions affect  $C_{\alpha}$  hydroxylation, but probably only to some extent. These ions however, are critical for the catalytic (deacetylation) reaction.