

**P016** The ground and excited state dynamics in the light-driven enzyme protochlorophyllide oxidoreductase is anchored by the multiple roles of active site Tyr and Lys residues  
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The light activated enzyme, protochlorophyllide oxidoreductase (POR), catalyses the *trans* addition of hydrogen across the C17-C18 double bond of protochlorophyllide (Pchl<sub>id</sub>), a key step in chlorophyll biosynthesis. Similar to other members of the short-chain alcohol dehydrogenase family of enzymes, POR has conserved Tyr and Lys residues in the active site domain (Tyr-X-X-X-Lys) with a proposed reaction mechanism involving Tyr hydroxyl group participation in the proton transfer. In the present work, we have analysed these active site residues of POR in detail by using site-directed mutagenesis to produce six single mutants (Y193F, Y193A, Y193S, K197A, K197R and K197Q). A combination of steady-state, laser photoexcitation and low temperature fluorescence measurements has revealed the importance of these residues in the overall catalytic cycle. We found that while none of the mutations completely abolished catalytic activity it appears that Tyr 193 and Lys 197 have multiple roles in the overall reaction pathway. Mutations to either of these residues impair the formation of the ground-state ternary enzyme-substrate complex, suggesting that they play an important role in providing interactions with the substrate molecules. In addition, by analysing the most active mutant, Y193F, we found that Tyr193 not only participates in the proton transfer step but is responsible for helping to stabilise the Pchl<sub>id</sub> excited state prior to catalysis, thus extending the lifetime of this precursor species. This work confirms the importance of these two residues in POR and suggests that they have multiple roles in both ground-state and excited-state processes during the reaction.