Substrate binding in Ascorbate Peroxidase

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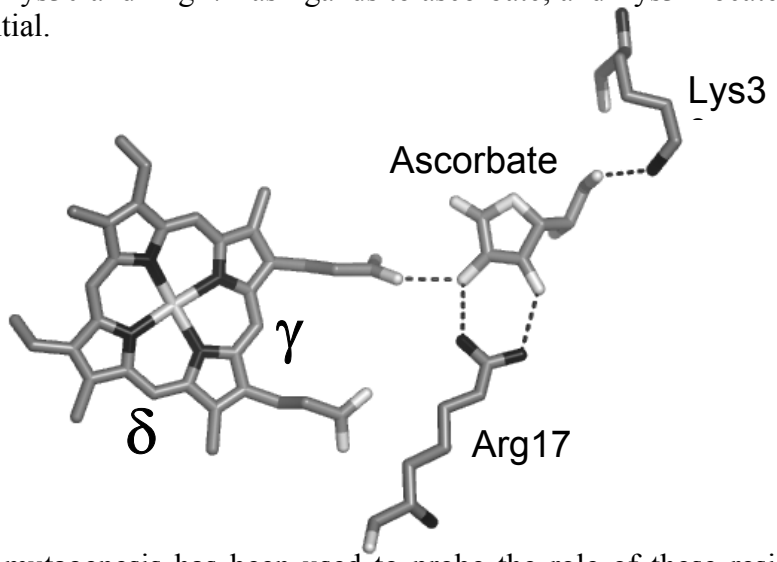
Ascorbate peroxidase is a haem-containing enzyme which catalyses the peroxide-dependent oxidation of ascorbate, and non-physiological aromatic substrates. Two, separate substrate binding locations have been identified: the first at the γ-heme edge, utilized by ascorbate; the second close to the δ-haem position, used by aromatic substrates. The crystal structure of the ascorbate peroxidase-ascorbate complex has recently been elucidated within our group1. The structure confirms Lys30 and Arg172 as ligands to ascorbate; and Lys31 located close enough to be possibly influential.



Site directed mutagenesis has been used to probe the role of these residues in ascorbate binding by ascorbate peroxidase, at the γ-meso site. The residues were mutated to alanine, aspartic acid and arginine/lysine using single, double and triple substitutions. The pre-steady state and steady state parameters of these mutants towards ascorbate and aromatic substrates have been determined, allowing us to assess the individual contributions to substrate binding of these residues.

1. Sharp, K. H.; Mewies, M.; Moody, P. C. E.; Raven, E. L., *Nature Struct. Biology*, 2003, **10**, 303-307