

# Origin of Stereospecificity of Enoylreductase Domains in Modular Polyketide Synthases

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In fully reducing modules of type I polyketide synthases (PKSs), the enoylreductase (ER) domain catalyses reduction of an enzyme-bound 2-enoyl thioester intermediate during polyketide biosynthesis. In the highly homologous fatty acid synthases, catalysis by the ER domain involves attack by a hydride ion, delivered from NADPH, on the  $\beta$ -carbon of the 2-enoyl intermediate, and protonation of the  $\alpha$ -carbon with a proton derived from the water of the reaction medium.<sup>1, 2</sup> In the fatty acid synthases, the saturated acyl product resulting from enoyl reduction is typically achiral, but in (for example) the methyl-branched fatty acyl components of mycobacterial cell walls, and in many reduced polyketides, reduction of the carbon-carbon double bond generates a stereocentre at C-2 which is either (*R*) or (*S*). We have found that for modular PKS ER domains, the configuration in the product correlates exactly with a single amino acid residue in the ER domain, which is systematically conserved but differs in the two outcomes. This allows a confident prediction of the configuration at such reduced centres in the product of newly identified PKS genes and enzymes. Modelling of ER domains on the enzymes of known structure revealed that this identifying residue lies at the active site. We have altered this residue by site-directed mutagenesis in a model PKS *in vivo*, and have obtained directed confirmation of its importance in determining the stereochemical course of ER-catalysed reduction.

1. B. Sedgwick, et al., *J.C.S. Chem Comm.*, 1980, **3**, 96-97

2. D. O'Hagan, *Nat. Prod. Rep.*, 1993, **10**, 594-624