

**P032** Modelling the reaction in citrate synthase reveals an unexpected mechanism

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Citrate synthase is an important model enzyme, which catalyses a challenging reaction. QM/MM modelling is providing key insights into its mechanism, tested by comparisons with experimental data (e.g. for alternative substrates). The reaction involves proton abstraction from acetyl-CoA to form an enolate (enolization) and nucleophilic attack of the resulting enolate on the carbonyl of oxaloacetate (condensation).

We have identified a new and unexpected mechanism for the reaction, from calculations which also highlight important shortcomings in standard modelling methods. Energy profiles for the two reaction steps were obtained using high-level (ab initio) QM/MM methods. The results confirm the existence of an acetyl-CoA enolate intermediate and the importance of the protein environment for stabilizing this species. When fluoroacetyl-CoA is used as substrate, the stability of the enolate explains the experimentally observed enantioselectivity.

Modelling of the condensation step reveals that it is concerted with an unusual proton transfer from an arginine residue to the carbonyl oxygen. Analysis of the contribution of individual residues in the active site highlights the importance of hydrogen bonds and salt-bridges in binding and stabilization. This mechanism is consistent with, and sheds new light on, experimental findings. It has important implications for other enzymes.