MACiE is a unique resource for understanding enzyme reaction mechanisms. It provides detailed chemical annotation of complete reactions, the constituent steps and the catalytic machinery. This is then integrated with information from collaborating databases, the available literature and cheminformatic analyses. MACiE’s multifaceted approach annotates enzyme mechanisms with greater precision than is currently available in any other individual enzyme database. Here, we present the latest release of MACiE which has been expanded to 350 entries, covering 90% of EC-sub-subclasses with available experimentally determined protein structures. This release of MACiE introduces new analysis tools. Overall reaction and step-wise chemical fingerprinting defines chemical centres more clearly and provides information about mechanistic similarity. In combination with the FunTree database, the evolutionary relationships between enzymes and the reactions they perform can be evaluated. Using MACiE’s integrative analysis the diversity of enzymes can be recorded both within and between superfamilies. In the case of the chloroperoxidases, for example, the alternative mechanisms of producing a hypohalous acid catalysed by three unrelated enzymes can be evaluated. Each utilises different catalytic CATH domains, amino acid residues and cofactors, signifying no apparent evolutionary relationship between the enzymes. The mechanistic differences are reflected in the composite bond change fingerprints, despite the overall reactions being identical. This latest release enhances MACiE’s ability to investigate the connections between enzyme structure, substrate specificity, active site architecture, cofactor dependence and chemical mechanism.