C–N Bond Forming Radical Rebound Is the Enantioselectivity-Determining Step in P411-Catalyzed Enantioselective C(sp^3)–H Amination: A Combined Computational and Experimental Investigation

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ABSTRACT: Engineered metalloenzymes represent promising catalysts for stereoselective C–H functionalization reactions. Recently, P450 enzymes were evolved to allow for new-to-nature intramolecular C(sp^3)–H amination reactions via a nitrene transfer mechanism, giving rise to diamine derivatives with excellent enantiocontrol. To shed light on the origin of enantioselectivity, a combined computational and experimental study was carried out. Hybrid quantum mechanics/molecular mechanics (QM/MM) calculations were performed to investigate the activation energies and enantioselectivities of both the hydrogen atom transfer (HAT) and the subsequent C–N bond forming radical rebound steps. Contrary to previously hypothesized enantioinduction mechanisms, our calculations show that the radical rebound step is enantioselectivity-determining, whereas the preceding HAT step is only moderately stereoselective. Furthermore, the selectivity in the initial HAT is ablated by rapid conformational change of the radical intermediate prior to C–N bond formation. This finding is corroborated by our experimental study using a set of enantiomerically pure, monodeuterated substrates. Furthermore, classical and *ab initio* molecular dynamics (MD) simulations were carried out to investigate the conformational flexibility of the carbon-centered radical intermediate. This key radical species undergoes a facile conformational change in the enzyme active site from the pro-(*R*) to the pro-(*S*) configuration, whereas the radical rebound is slower due to spin-state change and ring strain of the cyclization process, thereby allowing for stereoablative C–N bond formation. Together, these studies revealed an underappreciated enantioinduction mechanism for biocatalytic C(sp^3)–H functionalizations involving radical intermediates, opening up new avenues for the development of challenging asymmetric C(sp^3)–H functionalizations.