Microed Structure of a Protoglobin Reactive Carbene Intermediate

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In Microcrystal Electron Diffraction (MicroED) high-resolution structural data are collected from vanishingly small crystals, typically with a thickness in the nanometer range. By circumventing crystal size limitations, MicroED has the potential to enable studies of new and important chemical and biological structures which were previously beyond reach.
Taking advantage of recent technological advances in MicroED as well as in protein structure prediction, we have determined the first structure of an *Aeropyrum pernix* protoglobin (*Ape*Pgb), as well as a metallo-carbene intermediate. This *Ape*Pgb variant was obtained by directed evolution selecting for biocatalytic carbene transfer reactions. Since no wild-type structure was available as search model for the molecular replacement, the structure was phased using a predicted model generated by AlphaFold2. Our MicroED structure revealed how the introduced mutations enhance the carbene transfer activity by making the catalytic active site more accessible for the substrate. In addition, after exposing the tiny crystals to the substrate, we also trapped the reactive iron-carbenoid intermediate involved in this new-to-nature activity. The bound structure discloses how the enlarged active site pocket stabilizes the carbene bound to the heme iron and, presumably, the transition state for the formation of this key intermediate. This work demonstrates that with the recent improvements in MicroED technology, and the advancement in protein structure prediction, investigations of protein structures that were previously beyond technological reach are now possible.