

## **Macromolecular structure determination by micro-crystal electron diffraction**

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Micro-crystal electron diffraction (MicroED) has recently shown potential for structural biology. It enables macromolecular structure determination when only small micron-sized three-dimensional crystals are available that are beyond what can be resolved by conventional X-ray crystallography. However, up until now MicroED had only been applied to refine known protein structures that were already solved previously by X-ray diffraction. Here we present the first unknown protein structure of a novel R2lox metalloenzyme solved using MicroED. The structure was phased by molecular replacement using a search model of 35% sequence identity, and the resulting electrostatic scattering potential map at 3.0Å resolution was of sufficient quality to allow accurate model building and refinement. Our results illustrate that MicroED has the potential to become a widely applicable technique for protein structure determination, complementing X-ray crystallography when crystal volume is the limiting factor, with ongoing developments aimed at optimizing sample preparation and data acquisition, as well as investigating possible strategies for experimental phasing including isomorphous replacement and high-resolution imaging.