Direct electron detection, phasing and hydrogens

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Microcrystal electron diffraction (MicroED) uses electron cryo-microscopy (cryo-EM) for protein structure determination of crystalline samples too small for conventional X-ray crystallography [1]. Electrons interact with the electrostatic potential of the crystal, which means that scattered electrons carry information about the charged state of atoms and provide relatively stronger contrast for localizing hydrogen atoms. Identification of individual hydrogen atoms typically requires atomic resolution data and has thus far remained elusive for macromolecular MicroED. Here, we present the structure of triclinic hen egg-white lysozyme at 0.87 Å resolution using electron counting data collection on a direct electron detector at a significantly reduced exposure [2]. The low exposure ensures that the counts remain within the linear range of the camera, and reduces any negative effects of radiation damage to the structural integrity of the protein and the ability to localize hydrogen atoms. To enhance the signal at low exposure conditions we used focused ion-beam (FIB) milling to produce thin crystalline lamellae of an optimal thickness [3], combined with a slow rotation rate to systematically cover reciprocal space [2]. The structure was determined ab initio by placing an idealized helical fragment of three residues, followed by density modification and automated model building. Using the subatomic resolution data, we identified over a third of all possible hydrogen atoms in our structure from strong difference peaks, enabling direct visualization of hydrogen bonding interactions and the charged states of residues [4]. Furthermore, we find that the hydrogen bond lengths are more accurately described by the inter-nuclei distances than the centers of mass of the corresponding electron clouds. These results indicate a substantial improvement in MicroED data quality using electron counting, providing accurate intensities for phasing and visualizing hydrogen atoms and hydrogen-bond interactions in macromolecules.