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Phase Contrast Single Particle Analysis at Atomic Resolutions.

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Single particle analysis and X-ray crystallography are two standard approaches to answer many biological questions. However, X-ray crystallography has its own limitations due to non-sufficient crystal size or non-diffracted crystals. In this case single particle analysis become a strong complementary tool to deliver a wealth of structural information. In this approach the samples are highly purified in solution and plunge frozen in liquid ethane or ethane propane mixture which is cooled down by liquid nitrogen. Moreover, the samples embedded in a thin layer of vitreous ice leads to poor contrast due to low signal-to-noise ratio. There are two ways of generating phase contrast from the weak phase objects in their native and frozen hydrated states: one way is using the spherical aberration of the microscope lenses and intended defocus of the objective lens and the other way is using a phase plate. By using the phase plates, the low frequency information is well and continuously transferred to high frequency information, which is the strength of the phase contrast method compared to defocused based cryo-electron microscopy.

Among all types of phase plates, the so called Volta phase plate(VPP) is the most successful one which has shown its asset in the biological applications in both cryo-electron tomography and single particle analysis [1, 2, 3]. VPP is located in the back focal plane of the objective lens creating a phase shift between the unscattered and the scattered electron beams.

Since now the smallest protein complex which was studied by conventional single particle analysis is isocitrate dehydrogenase with a molecular size of 93 kDa at 3.8 Å resolution [4]. Therefore, with the current status of the conventional single particle analysis there are some remained challenging protein complexes which could be analysed by using the VPP. In this work, phase contrast single particle analysis is shown for structure determination of a human haemoglobin with a molecular weight of 64 kDa at 3.2Å resolution (Figure 1) [5]. This suggests a benefit of using the VPP technology for important and challenging drug targets in structure-based drug design.

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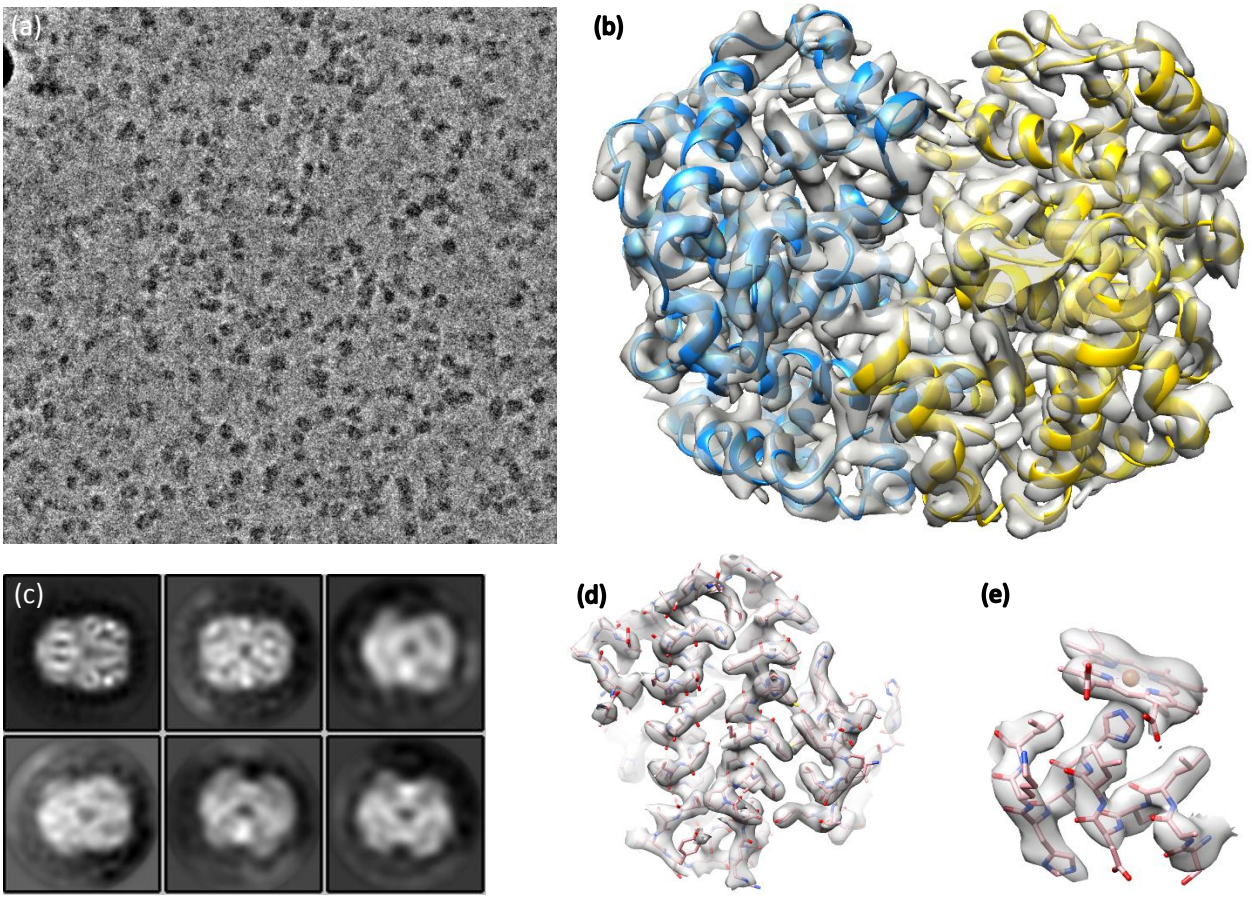

Figure 1. VPP structure of human haemoglobin at 3.2 Å resolution. (a) A VPP micrograph of haemoglobin, defocus -500 nm. (b) 3D reconstruction and fitted model of haemoglobin. (c) representative 2D class averages of haemoglobin. (d,e) coverage of side chains and haem group from reconstructed 3D map. Experimental conditions: Titan Krios 300kV, zero-loss energy filtering, K2 Summit direct detector; Pixel size: 0.52 Å.

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