ATP-BOUND STATES OF GroEL AND GroES CAPTURED BY CRYO-EM AND SINGLE PARTICLE IMAGE PROCESSING

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The molecular chaperones GroEL and GroES facilitate the correct folding of substrate proteins both in vitro and in vivo, in an ATP-dependent manner. The effects of ATP binding in the GroE system have been examined by cryo-electron microscopy and single particle image processing. Domain rearrangement in several ATP-bound GroE complexes have been characterized by fitting of atomic coordinates for the GroEL domains into EM-density as rigid bodies. ATP-binding causes the intermediate domain of GroEL to rotate downward by 20°. This rotation causes switch in the pattern of inter-subunit salt bridge interactions within the GroEL ring. The broken salt bridge interaction frees the GroEL apical domains to undergo a 25° anti-clockwise twist, which partially buries the substrate binding site. The newly formed salt bridge provides a pathway for cooperative communication of ATP binding between ATP binding sites. Small rotations of the GroEL equatorial domains disrupt the ring-ring interface and suggest a model for negative cooperative signal transduction in GroEL. The effects of ATP binding to the open ring of a GroEL-GroES complex have also been examined. In this case we propose that a strained conformation is adopted which provides the physical basis for ejection of GroES from the opposite ring.

Keywords: CRYO-EM, SINGLE PARTICLES, MOLECULAR CHAPERONES

OBSERVING METASTABLE STATES USING TIME RESOLVED HIGH TEMPERATURE DIFFRACTION DATA

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Advances in 3rd generation synchrotron sources now provide sufficient photon flux at high energies (> 40 keV) to measure fully refinable x-ray diffraction pattern in under a few seconds. Precise structural information can be obtained from the refinements. Using high-energy photons (up to 125 keV) and area detectors, we have investigated the solid-state phase transformations in a number of viterous (Ti-Zr-Cu-Ni, Zr-Cu-Ni-Al, Zr-Pd-Cu) and partially vitreous (Nd-Fe-B) intermetallic alloys at the MUCAT and SRI-CAT beamline at the Advance Photon Source. Utilizing high-energy photons and precise temperature control up to 1750 K, it is now possible to obtain structural investigations is the intense X-ray absorption of cell walls, electrolytic solution and sample itself, so that rather weak diffraction signals are usually obtained even at synchrotron facilities. To overcome this problem, diffraction by high-energy X-rays, both monochromatic (conventional Angular Dispersive modality) and polychromatic (Energy Dispersive modality) can be utilized. Two examples will be shown: The study of the lattice parameter variation of the spinel Li2MnO4, by means of the 87.5 keV X-ray beam available at ID15B (ESRF, Grenoble). This compound is defined as “zero-strain” because of the minimal changes (±0.1%) it exhibits when used as anodic material. Such a high structural stability is considered as the main reason for the long cycle life of this compound. The result was that the variations are qualitatively similar to those of common electrode materials, although on a much smaller scale. The study of LiNi0.8Co0.2O2 cathodic material, used in commercial Li-ion batteries. A laboratory Energy Dispersive diffractometer that makes use of a polychromatic X-ray beam reaching 60 keV was utilized and real-time phase transitions upon cycling were observed.

Keywords: HIGH ENERGY DIFFRACTION, IN SITU, LI-ION BATTERIES