

ATP-BOUND STATES OF GroEL AND GroEL-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 vitreous (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 information at heating rates on par with thermal analysis (5 to 50 K/min). Various experimental set-ups will be presented to show how to obtain good S/N for rapid data acquisition suitable for Rietveld refinement and higher wave momentum experiments necessary for atomic pair distribution function analysis. Examples of 'hidden' metastable phases during devitrification of metallic glass alloys will be presented.

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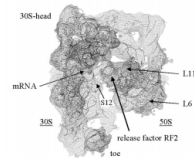
Keywords: POWDER DIFFRACTION DEVITRIFICATION METALLIC GLASS

RELEASE FACTOR - RIBOSOME INTERACTIONS REVEALED BY COMBINATION OF CRYO-ELECTRON MICROSCOPY AND CRYSTALLOGRAPHY

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This study aims at understanding the structure-function relationship of functional complexes involved in the termination of peptide synthesis. Termination occurs in response to a stop codon in the mRNA rather than a sense codon moving into the ribosomal aminoacyl site. In prokaryotes, two protein factors called RF1/RF2 and RF3 are required: binding of RF1/RF2 to the ribosome induces hydrolysis of the peptidyl-tRNA; binding of RF3 induces release of RF1/RF2, followed by GTP-hydrolysis triggering the release of RF3. We have determined the structure of the E. coli 70S - RF2 complex to 12Å resolution using cryo-electron microscopy and angular reconstitution of single particles. Analysis of these data and comparison with the crystal structures of the 70S ribosome from T. thermophilus and the E. coli release factor RF2 was performed through manual and numerical fitting in reciprocal space. Beyond a localization of the factor, the fit allows molecular details of translation termination to be revealed. RF2 decodes the stop codon through direct interactions with the mRNA. RF2 bridges the intersubunit space from the 30S to the 50S subunit thus transmitting the decoding signal to the peptidyl transferase center through interactions with 50S RNA and proteins. Binding of RF2 induces long-range conformational changes of the ribosome. An additional reconstruction concerning the RF3 ribosomal complex discloses the binding site of the factor, which is compatible with a pre-bound RF2. The data allow to localize the GTPase domain and map the interface between the subunits of the RF2/RF3 heterodimer that forms on the ribosome.



Keywords: RIBOSOMAL RELEASE FACTOR, CRYO-ELECTRON MICROSCOPY, FITTING OF CRYSTAL STRUCTURES

HIGH-ENERGY X-RAY DIFFRACTION STUDIES OF BATTERY MATERIALS BY POLYCHROMATIC AND MONOCHROMATIC RADIATION

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Li-ion batteries are the most common rechargeable power supply for portable electronic devices. Their electroactive materials are capable of reversibly inserting (de-inserting) Li⁺ in their crystalline structure upon reduction (oxidation). The observation of the changes that accompany this process is fundamental to understand the reasons of the progressive decrease in the battery capacity. To follow these changes the in-situ X-ray diffraction performed during cycling is generally used. The main obstacle for such 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 Li_{4/3}Ti_{5/3}O₄, 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 ($\Delta a/a < 1\%$) 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 LiNi_{0.8}Co_{0.2}O₂ cathodic material, used in commercial 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