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The maturation pathway of flaviviruses studied by crystallography and electron microscopy

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Many viruses go through a maturation step in the final stages of assembly before being transmitted to another host. The maturation process of flaviviruses is directed by the proteolytic cleavage of the precursor membrane protein (prM), turning inert virus into infectious particles. We have determined the crystal structure of a recombinant protein in which the dengue virus prM is linked to the envelope glycoprotein E. The structure represents the prM-E heterodimer and fits well into the cryo-electron microscopy density of immature virus at neutral pH. The pr peptide beta-barrel structure covers the fusion loop in E, preventing fusion with host cell membranes. The structure provides a basis for identifying the stages of its pH-directed, conformational metamorphosis during maturation, ending with release of pr when budding from the host.

Keywords: virus structure, flaviviruses, maturation

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Structural insights into molecular chaperone activity

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Anfinsen established that the polypeptide sequence dictates the folded conformation of a native protein. Protein folding in the crowded cellular milieu is often frustrated by off-reaction aggregations, however, and especially so under destabilizing stresses such as thermal shock. Moreover, the native state may require an intimate co-folding with other components for assembly into multimeric complexes. Molecular chaperones, many of which are heat shock proteins (Hsps), provide a machinery to assist protein folding in the cell. We present studies on two kinds of molecular chaperones. Trigger factor (TF) is a molecular chaperone that associates with bacterial ribosomes, where it is thought to assist in the folding of nascent polypeptides. We also find that ribosome-free TF stably associates with a large repertoire of full-length proteins, including ribosomal protein S7. The crystal structure of a TF:S7 complex from T. maritima reveals the molecular basis of promiscuous substrate recognition by TF, indicates how TF could accelerate protein folding, and suggests a role for TF in the biogenesis of ribosomes and other protein complexes. Hsp70 chaperones use ATP-driven cycles of binding and release of unfolded polypeptides to assist in diverse processes of protein folding and translocation. We deduced hypotheses about the mechanism of Hsp70 chaperone activity from the crystal structure of an ATP complex of yeast Sse1, an Hsp110 chaperone from the Hsp70 superfamily. Mutational tests in Hsp70 chaperones yeast Ssa1 and E. coli DnaK define an Hsp70 chaperone cycle in which radically different Hsp70 conformations are engaged during the chaperone cycle. Allosteric coupling between the ATP and polypeptide binding sites promotes disaggregation and protein folding.

Keywords: conformational change, protein folding, MAD phasing

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Structures of the ribosome on different functional states

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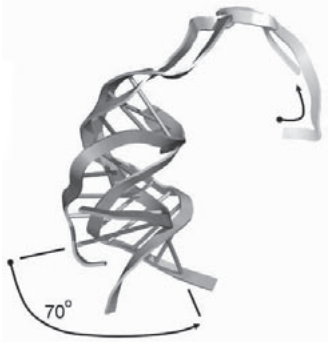
Recent crystal structures of bacterial 70S ribosome containing functional ligands provided information about the general organization of the ribosome and its functional centres. Ribosomes co-crystallized with messenger RNA (mRNA) containing strong Shine-Dalgarno (SD) sequence and transfer RNAs (tRNA) have shown diffraction to 2.8Å resolution. This ribosome complex with initiator tRNA in peptidyl-tRNA binding site represents functional state of translation initiation. We have compared x-ray structures of ribosome complexes modelling translation initiation, post-initiation and elongation states. In the initiation and post-initiation complexes, the presence of the SD duplex causes strong anchoring of the 5 ´ -end of mRNA on the platform of the 30S subunit, where numerous interactions between mRNA and the ribosome take place. Conversely, the 5 ´ -end of the elongator mRNA lacking SD interactions is flexible during elongation. The post-initiation ribosome complex reveals that after initiation of translation, while SD interaction is still present, mRNA moves in the 3´-5´ direction with simultaneous clockwise rotation and lengthening of the SD duplex (Figure).



Keywords: ribosome structure, functional complexes, mRNA and tRNA

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Structural basis of transcription: Structures of the bacterial RNA polymerase elongation complexes

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Understanding the mechanisms of transcription elongation and its regulation requires detailed structural information. The structure of the bacterial EC (2.5Å) revealed the post-translocated intermediate

with the DNA template in the active site available for pairing with the substrate. DNA strand separation occurs one position downstream of the active site, implying that only one substrate at a time can bind to the EC. At the upstream edge of the RNA/DNA hybrid, the first displaced RNA base is trapped within a protein pocket, suggesting a mechanism for RNA displacement. The displaced RNA resides in the RNA exit channel and adopts a conformation mimicking that of a double helix, providing insight into a mechanism for hairpin-dependent pausing and termination. The mechanism of substrate loading in multi-subunit RNA polymerases is crucial for understanding the general principles of transcription. We have determined the EC structures (3Å) with a non-hydrolysable substrate analogue (AMPcPP), and with AMPcPP plus the antibiotic streptolydigin (Stl). In the EC/AMPcPP structure, the substrate binds to the active ('insertion') site closed through refolding of the trigger loop (TL). In contrast, the EC/AMPcPP/Stl structure reveals an inactive ('preinsertion') open substrate intermediate stabilized by Stl-induced displacement of the TL. Our data suggest three main implications. First, the two-step preinsertion/insertion mechanism of substrate loading may be universal for all RNA polymerases. Second, freezing of the preinsertion state is an attractive target for the drug design. Last, the TL emerges as a regulatory target whose refolding can be modulated by transcription factors.

1. Vassylyev et al. (2007a) Nature, 448, 157-162.
2. Vassylyev et al. (2007b) Nature, 448, 163-168.

Keywords: RNA polymerase, crystal structure, elongation complex

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Ligand-induced structural changes of giant hemoglobin

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Hemoglobin (Hb) is a major protein that transports oxygen in many animals. Mammalian tetrameric Hb is an allosteric protein that has been extensively studied for a century. In contrast to mammalian or vertebrate tetrameric Hb, some of the annelids have extracellular giant Hbs of 3,600 kDa or 400 kDa. These giant Hbs have remarkably different quaternary structures and oxygen binding properties. Recent crystallographic studies have revealed the structures of both 3,600 kDa and 400 kDa Hbs [1], and their common quaternary structure of dodecameric subassembly composed of four kinds of globin subunits. All of these structures were solved with oxygenated or CO-liganded forms at low or moderate resolution, and the unliganded form of these giant Hbs had remained unknown. To elucidate cooperative mechanisms of the giant Hbs in detail, we have determined crystal structures of 400 kDa Hb of *Oligobrachia mashikoi* (a frenulate beard worm) at over 2 Å resolution. The obtained structures include partially unliganded met forms in which three of four globin subunits in the 24mer assembly [2]. Remarkable structural changes at the AB loop regions in all subunits are seen between the oxygenated form and the partially unliganded form. These movements cause quaternary rearrangements of the dimer and the dodecamer subassemblies of the giant Hb. These results suggest that the ligand-induced structural changes of *Oligobrachia* Hb are quite different from those of the well-studied Hbs.

[1] Numoto N., et al., *Proc. Natl. Acad. Sci. USA*, 2005, **102**, 14521-14526. [2] Numoto N., et al., *Proteins*, 2008, in press.

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Keywords: hemoglobins, cooperative, ligand-protein interactions

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Recent developments in GISAXS and GISANS - nanobeams and *in-situ* kinetic investigations

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Grazing incidence small angle scattering with x-rays (GISAXS) and neutrons (GISANS) are advanced methods to probe structures from the molecular level up to micrometer scale [1]. The grazing incidence condition enables a tunable surface sensitivity and thus to distinguish surface from volume structures in thin films. With micro- and nano-beams a local structure becomes accessible. Comparable to scanning probe techniques, the combination of small x-ray beams with scanning of the sample position relative to the beam allows for probing position dependent structures. Beam size, step size and resolution are relevant experimental parameters in terms of characterization of areas, domains. In-situ kinetic investigations allow to access the observation of morphological changes in thin films. Within this presentation several examples are discussed to demonstrate the actual possibilities of these techniques.

[1] P. Muller-Buschbaum: Structure determination in the thin film geometry using grazing incidence small angle scattering; in Polymer Surfaces and Interfaces: Characterization, Modification and Applications, edt. M. Stamm, p. 17-46, Springer Berlin, ISBN-13: 978-3-540-73864-0 (2008)

Keywords: GISAXS, polymer films, nanostructures

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Quick X-ray reflectometory in simultaneous multiwavelength dispersive mode

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For recording specular X-ray reflectivity curve on the subsecond to millisecond timescales, the entire profile of the reflectivity curve of interest is measured with the geometry shown in the figure. A horizontally convergent X-ray beam which has a one-to-one correlation between its direction and energy is realized when a quasi-parallel white X-ray beam is incident on and diffracted by a curved crystal. The X-ray beam is then incident on the surface of the