

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 (StI). 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/StI structure reveals an inactive ('preinsertion') open substrate intermediate stabilized by StI-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

MS.01.5

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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 *Oligobranchia 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 *Oligobranchia* 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*, ed. M. Stamm, p. 17-46, Springer Berlin, ISBN-13: 978-3-540-73864-0 (2008)

Keywords: GISAXS, polymer films, nanostructures

MS.02.2

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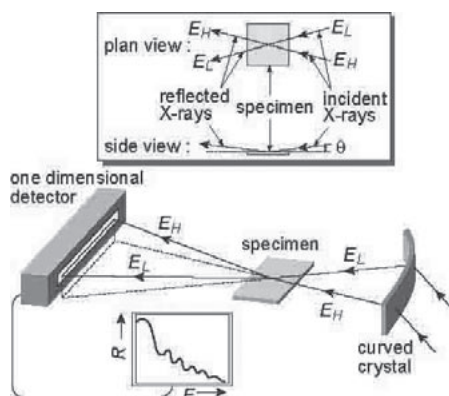
Quick X-ray reflectometry in simultaneous multiwavelength dispersive mode

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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

specimen placed at the focus in a geometry that the glancing angle in the vertical direction is the same for all the X-ray components, and they are reflected in the vertical direction by the surface and diverge in the horizontal plane. The perpendicular momentum transfer, given by $q=4\pi\sin\theta/\lambda$, continuously changes as a function of the ray direction even with the fixed glancing angle θ since the wavelength (energy) λ changes. The X-ray intensity distribution across the beam direction measured downstream of the specimen using a one-dimensional detector represents the X-ray reflectivity curve. Some results of static and dynamic measurements will be reported, and the characteristics and potentials of the method will be discussed.



Keywords: specular X-ray reflectivity, time-resolved, simultaneous multiwavelength dispersive

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Structural characterization using the multiple scattering effects in GISAXS

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The multiple scattering effects present in grazing-incidence small-angle X-ray scattering (GISAXS) data and interference between them are addressed theoretically as well as experimentally with measurement of a series of patterns at different incident angles, referred to as 'incident-angle-resolved GISAXS' (IAR-GISAXS). X-ray reflectivity (XR), GISAXS and IAR-GISAXS of virus particles on Si-substrate supported-polystyrene films have been measured and compared. It was found that under certain conditions it is possible to extract the correct structural features of the materials from the GISAXS/IAR-GISAXS data using the kinematic SAXS formalisms. Furthermore, the Kiessig fringes in GISAXS enable the measurement of the average distance between the particle and the substrate, similar to the measurement of film thickness using the fringes in the XR data.

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Keywords: GISAXS, reflectivity, virus assembly

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Neutron reflectively study of chain conformation in polyelectrolyte brushes at the liquid interface

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The zwitterionic and cationic polyelectrolyte brushes on the quartz plates were prepared by surface-initiated atom transfer radical polymerization of 2-methacryloyloxyethyl phosphorylcholine (MPC) and N,N-dimethylaminoethyl methacrylate, which was successively converted to methacryloyloxyethyl trimethylammonium iodide (META1) by quaternization with methyl iodide. Neutron reflectivity (NR) was measured by irradiation of neutron beam from the quartz plate to the interface between liquid phase and immobilized polyelectrolyte brush. NR profiles of polyelectrolyte brush/D₂O interface indicated that the polymer chains exhibited stretched conformation and formed concentration gradient of D₂O under swelling states. The effects of ionic strength on the conformation and surface properties of densely grafted polyelectrolyte brush were analyzed by NR. The difference between reflectivity of polyelectrolyte brushes in D₂O and in salt solution was negligible. The authors supposed that quaternary ammonium iodide at the side chain of poly(META1) chains are bulky and the mobility of iodide ion is limited because of weak hydration. On the other hand, PMPC brush slightly shrunk at the EtOH/Water in cononsolvency region.

Keywords: polymer brush, water interface, polyelectrolyte

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Following growth and catalytic reaction of oxide supported metal nanoparticles with GISAXS

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X-ray scattering techniques using the grazing incidence geometry offer the opportunity to probe in situ the crystallographic structure and the morphology of metal nanoparticles grown on oxide planar surfaces. Combining wide angle (GIXD) and small angle scattering (GISAXS) can give some insights into connected thermodynamic and kinetic parameters of metal/support interface, namely the role of stress due the lattice mismatch on the epitaxial orientation, the adhesion/wetting properties, the particle equilibrium shape and the peculiarities of the growth modes and the chemical reactivity. One key advantage of X-ray is the capability to perform measurement not only during particle growth under vacuum conditions but also at high pressure during the course of an actual catalytic reaction. This will be illustrated with our recent results on Au/TiO₂(110), a model planar system of gold-based catalyst. Among others, the following points will be discussed: