MS6-O5 Cooperative Motion Analysis of group II chaperonins at single molecule level using nanocrystal and time-resolved diffraction measurement

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Group II chaperonin, found in archaea and in the eukaryotic cytosol, is an indispensable protein that captures a nonnative protein and refolds it to the correct conformation in an ATP dependent manner. ATP-induced structural changes are essential for chaperonin activity and we had reported that the diffracted X-ray tracking (DXT) method could trace ATP induced conformational change of group II chaperonin at single molecule level (Sekiguchi et al., PLoS ONE 2013). In DXT, nanocrystal immobilized on one side of chaperonin-ring is used as motion tracer for structural change of chaperonin as shown in the figure. In this study, we analyzed how ATPase deficient mutant modulate dynamic motion of chaperonin, and cooperativity inter and intra communication of chaperonin ring. We found that although one ring of the asymmetric ring complex lacks ATPase activity, the other wild-type ring undergoes an ATP-dependent conformational change and maintains its protein-folding activity. The results clearly demonstrate that inter-ring communication is dispensable in the reaction cycle of group II chaperonins (Yamamoto et al., JMB 2014), despite the reaction cycle of group I chaperonins, for example GroEL-ES system in E-coli, is controlled by inter-ring allosteric communication.

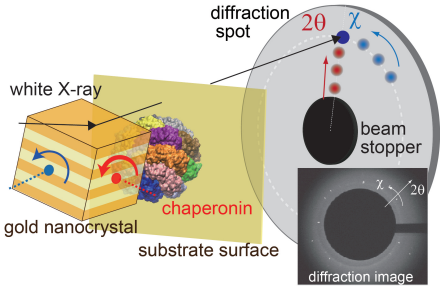

Figure 1. Schematic illustration of motion analysis of chaperonin by diffracted X-ray tracking.

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MS7-O1 Structures of lytic polysaccharide monooxygenases and their interaction with polysaccharide substrates

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The recent discovery of lytic polysaccharide monooxygenases has revolutionized our understanding of how polysaccharides are degraded in nature and our thoughts on how they could be most efficiently degraded in industrial processes. Cellulose, the main constituent of the plant cell wall, is the most abundant polysaccharide on earth, and as it is present in all plants is of particular of interest for the production of second generation biofuels. Conventional cellulases are glycoside hydrolases, and catalyze the cleavage of the glycosidic linkage by using water molecule in mechanisms involving one or more carboxylate residues. In contrast, lytic polysaccharide monooxygenases (LPMOs) are metalloenzymes which cleave the glycosidic linkage by activating molecular oxygen through a mononuclear copper center held by the so-called histidine brace, thereby mediating oxidation at C1 or C4 (1,2).

Carbohydrate modifying enzymes are classified in the Carbohydrate Active Enzyme Database, CAZY, where LPMOs are now classified as Auxiliary Activities (3) in families AA9 (GH61 prior to discovery of their LPMO activity (4)), AA10 (CBM33 prior to discovery of their LPMO activity), AA11 and AA13. The initially characterized LPMOs had activity on the insoluble β-1,4-linked polysaccharides chitin and cellulose, but now LPMOs active on soluble β-1,4-linked oligo-/polysaccharides substrates, as well as active on α-1,4-linked polysaccharides, have been both characterized biochemically and structurally (5-7).

This talk will provide an overview on the structural biology of LPMOs so far and focus primarily on substrate binding and specificity, in particular the structural differences between α-1,4 and β-1,4- active LPMOs (5) and the very first LPMO substrate complexes which we recently obtained (7).

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1. Vaaje-Kolstad et al. (2010) *Science* **330**:219-222.
2. Quinlan et al. (2011) *PNAS* **108**:15079-15084.