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The chaperonin GroEL adopts a double-ring structure with various modes of allosteric communication. The simultaneous positive intraring and negative inter-ring co-operativities alternate the functionality of the folding cavities in both protein rings. Mutation of glutamic acid 434 to lysine, a residue located at the rings interface, alters the negative inter-ring co-operativity [1]. The crystal structure of the mutant chaperonin GroEL<sub>E434K</sub> has been determined at low-resolution (4.5 Å) and compared with two other structures: the wild-type GroEL [2] and the allosteric-defective GroEL<sub>E461K</sub> [3] mutant. Despite the allosteric-defective behavior of GroEL<sub>E434K</sub>, its structure remains strikingly similar to that of the wild type GroEL.

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### Structure determination of toxin complex from *Clostridium botulinum* serotype D

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*Clostridium botulinum* produces seven immunologically distinct serotypes of neurotoxin (BoNT; 150 kDa), classified as A to G. BoNT forms a large toxin complex (TC) varying from 300 kDa up to 900 kDa, through association with non-toxic non-hemagglutinin (NTNHA; 130 kDa) and three kinds of hemagglutinin (HA) subcomponents, HA-70, HA-33 and HA-17 (70, 33 and 17 kDa, respectively). Recently, we proposed a hypothetical 14-mer subunit model of the botulinum serotype D TC on the basis of the transmission electron microscopy (TEM) observations, X-ray crystallography and biochemical analyses [1]. TEM images revealed an ellipsoidal-shaped structure with three-arms attached. The crystal structure of HA-33/HA-17 complex was determined first by the molecular replacement (MR) method, and that of BoNT was generated by the homology modeling using serotypes A and B BoNTs as templates. Following our novel model, Nakamura *et al.* solved the trimer-like structure of HA-70 subcomponent [2]. Thereby, only the structure of NTNHA is remaining unknown at present. Crystals of the M-TC, a complex of BoNT and NTNHA, was obtained before [3]. The crystals diffracted to 8 Å resolution and were not suitable for structure analysis. Unfortunately, no crystals could be obtained ever again.

In the present study, the crystal structure of the HA-33/HA-17 complex was determined again by the SIRAS method using a platinum derivative to confirm the structure resulted from the MR method. All diffraction data were collected on an in-house X-ray system; a Rigaku R-Axis VII image plate detector equipped with a Rigaku FR-E+ SuperBright X-ray generator. The platinum derivative was obtained by soaking crystals in 10 mM K<sub>2</sub>PtCl<sub>4</sub> solution containing 7% PEG8000, 0.1 M MES (pH 6.5) and 0.1 M MgCl<sub>2</sub> for 10 min at room temperature.

Native and derivative data sets were collected to 2.15 and 2.30 Å resolution, respectively, at 93K. The final structure was refined to R<sub>crist</sub> of 20.3% and R<sub>free</sub> of 24.7% with one heterotrimeric HA-33/HA-17 complex in the asymmetric unit. The SIRAS structure confirmed that the lower electron density of HA-17 is not due to the phasing bias originated from the MR method but due to its flexible structure.

Our ultimate goal is to conclude the unique subunit structure of botulinum TC by combining the various techniques. As one of new attempts, we are trying to elucidate the three-dimensional shape of the NTNHA protein or complexes including NTNHA and the arrangement of each subcomponents in the TC using biological small angle X-ray scattering (BioSAXS). In addition, the measurements of protein-protein interaction in solution by quartz crystal microbalance (QCM) technique are currently in progress.

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### Structural insights into a dodecameric machine – The RuvBL1/RuvBL2 complex

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RuvBL1 (RuvB-like 1) and its homolog RuvBL2 are evolutionarily highly conserved AAA<sup>+</sup> ATPases essential for many cellular activities. They play an important role in chromatin remodeling, transcriptional regulation and DNA damage repair. RuvBL1 and RuvBL2 are overexpressed in different types of cancer and interact with major oncogenic factors, such as β-catenin and c-myc regulating their function. We solved the first three-dimensional crystal structure of the human RuvBL complex with a truncated domain II and show that this complex is competent for helicase activity. The structure reveals a dodecamer consisting of two heterohexameric rings with alternating RuvBL1 and RuvBL2 monomers bound to ADP/ATP, that interact with each other via the retained part of domain II. Interestingly, truncation of domain II led to a substantial increase in ATP consumption of RuvBL1, RuvBL2 and their complex. In addition, we present evidence that DNA unwinding of the human RuvBL proteins can be autoinhibited by domain II, which is not present in the homologous bacterial helicase RuvB. Our data give new insights into the molecular arrangement of RuvBL1[1] and RuvBL2 and strongly suggest that *in vivo* activities of these highly interesting therapeutic drug targets are regulated by cofactors inducing conformational changes via domain II in order to modulate the enzyme complex into its active state.

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