1P029**Structure of 6-Pyruvoyl Tetrahydrobiopterin Synthase from *Pyrococcus horikoshii* OT3: Novel Oligomerisation and Substrate Binding Modes**○Bagautdin Bagautdinov¹, Mitsuki Sugahara², Naoki Kunishima¹¹Advanced Protein Crystallography Research Group, RIKEN SPring-8 Center, Harima Institute, ²Structural Biophysics Laboratory, RIKEN SPring-8 Center, Harima Institute

6-Pyruvoyl tetrahydrobiopterin synthase (PTPS) catalyses the conversion of dihydroneopterin triphosphate to 6-pyruvoyl tetrahydropterin, in the second of three enzymatic steps synthesis of tetrahydrobiopterin (BH4) from guanosine triphosphate (GTP). The pathway product, BH4 is an essential cofactor for several aromatic aminoacid monooxygenases and the nitric oxide synthases. The only known crystal structure of PTPS from rat liver composed of a pair of trimers arranged in a head-to-head fashion to form the functional hexamer with active site close to trimer-trimer interface (Ploom et al., J. Mol. Biol. 286, 851). We determined the crystal structure of PTPS from the hyperthermophilic archeon *Pyrococcus horikoshii* OT3 (PhPTPS) in native form and in complex with pterin at 2.1 and 2.2 Angstrom resolution respectively. The tertiary structure of PhPTPS is found as trimer but packing is different from head-to-head mode characteristic for rat liver PTPS and additionally the two structures show substantial folding deviations in the N-terminal part. We demonstrate, that beside the novel trimeric oligomerisation pattern, PhPTPS possesses unique mechanism of substrate attraction and orientation. We propose that bacterial PhPTPS acts as a functional analogue of PTPS from higher order organisms, but oligomeric state and active site architecture may be evolutionally diverged.

1P031**Mail-in data collection at SPring-8 protein crystallography beamlines**○Nobuo Okazaki¹, Kazuya Hasegawa¹, Go Ueno², Yuji Fukumoto², Hironori Murakami², Hisanobu Sakai¹, Masaki Yamamoto^{1,2}¹SPring-8/JASRI, ²SPring-8/RIKEN

Mail-in data collection is a new service at synchrotron facilities in which beamline operator conduct data collection for samples sent from researchers by home-delivery services. It's a quite convenient system for most users because they can conduct experiments with synchrotron radiation without visiting. Here in SPring-8, we have already started the mail-in data collection at protein crystallography beamlines. The mail-in data collection for academic users has been routinely operated at BL26B2 since September 2005. The commercial one targeting researchers at pharmaceutical companies has started in July of this year, which works by the joint management of SPring-8 and private incorporations for the expansion of user and is based on this system.

Our system makes use of automated beamline operation using sample changer SPACE and beamline control software BSS. The experiment at beamline is carried out by two-step operation modes that are crystal evaluation in day time and automatic data collection in night. Diffraction data are collected only for superior crystals in the second step. Determination of experimental conditions and selection of crystals based on evaluation data are left to users. Newly developed system D-Cha which is made up of relational database and web interface enables registering measurement conditions and observing the recorded images at distant place. Therefore, the SPring-8 mail-in system can be characterized as a kind of remote access data collection with the support of beamline operators.

1P030**Homodimeric Crossover Structure of the Human GCSF-Receptor Signaling Complex**○Taro Tamada¹, Eijiro Honjo¹, Yoshitake Maeda², Tomoyuki Okamoto², Matsujiro Ishibashi³, Masao Tokunaga³, Ryota Kuroki¹¹Quantum Beam Sci., Japan Atomic Energy Agency, ²Pharm. Research Lab., Kirin Brewery Co. Ltd., ³Faculty of Agri., Kagoshima Univ.

Granulocyte colony-stimulating factor (GCSF) has become an important cytokine for medical treatment of patients suffering from granulopenia through regulating the maturation, proliferation, and differentiation of the precursor cells of neutrophilic granulocytes. Binding of GCSF to the extracellular Ig-like and CRH domain of its receptor (GCSF-R) triggers receptor homodimerization, resulting in activation of JAK-STAT type signaling cascades. The stoichiometry of the GCSF/GCSF-R complex has been a matter of some debate, with various proposed values (1:1, 2:2 and/or 4:4). Here, we report the crystal structure of a complex between human GCSF (hGCSF) and the Ig-like and CRH domains of human GCSF-R (hGCSF-R) at 2.8 Å resolution. The signaling 2:2 complex is formed via cross-over interactions between the Ig-like domain of hGCSF-R and the neighboring hGCSF, forming a two-fold axis of crystallographic symmetry. This conformation is quite different from that of the heterogeneous mGCSF-R complex, and more closely resembles the 2:2:2 active assembly of human interleukin-6 (IL-6), human IL-6 α-receptor and human gp130 (which is a shared signal transducing receptor for several cytokines), and the 2:2 assembly of viral IL-6 and human gp130. The Ig-like domain cross-over structure necessary for GCSF-R activation is consistent with previously reported thermodynamic and mutational analyses.

1P032**Real-Time SAXS Observation of Assembling-Disassembling Cycles of Cyanobacterial Circadian Clock Proteins**○Atsushi Nohara¹, Kazuki Ito², Yuichiro Maeda¹, Takao Kondo¹, Shuji Akiyama³¹Division of Biol. Science, Graduate School of Science, Nagoya University, ²RIKEN Harima Institute SPring-8 Center, ³PRESTO, Japan Science and Technology Agency

Three circadian clock proteins, KaiA, KaiB and KaiC, constitute a self-sustained circadian clock in cyanobacteria. Oscillation of KaiC phosphorylation can be reconstituted *in vitro* by incubating KaiC with KaiA, KaiB and ATP (Nakajima *et al.* 2005). While the importance of intermolecular interactions among the Kai proteins is well documented, there are no real-time and quantitative observations of the assembling-disassembling cycle in solution.

To take a structural snapshot of dynamic processes in real time, we followed the oscillatory reaction of the Kai proteins using small-angle x-ray scattering (SAXS). We found a global and circadian oscillation of the SAXS curves. Weight-averaged molecular weight (MW) calculated from the forward scattering intensity ($I(0)_{app}$) oscillated in the range from 400 to 460 kDa with a period of about 22 hr. $I(0)_{app}$ was peaked during the dephosphorylation process of KaiC (at IT 36 hr), and subsequently decreased after switching of KaiC from the dephosphorylation- to phosphorylation-phases. These results indicate that the Kai proteins form a large molecular complex to promote the dephosphorylation of KaiC.

Molecular size and components of the Kai complexes were inspected by fitting the oscillation pattern of $I(0)_{app}$ to several hypothetical models. If we assume a fraction of free KaiC to be about 0.4 (Kageyama *et al.* 2006), MW of the largest complex was estimated to be roughly 800kDa. Based on these estimations, we will discuss the kinds and numbers of protein components in the Kai complex.