

Structural Biology of Signal Transduction Molecules

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In order to understand the mechanism of intracellular signaling at molecular basis, we started to analyze the structure of molecules and domains that play roles in signal transduction using x-ray crystallography.

At Spring8, we have collected data sets of one protein and two domains crystals. First, data sets of native(Zn^{2+}) and Hg soaked crystals of Beta2-Chimaerin were collected. Beta2-Chimaerin consists of two domains, C1(DAG binding) domain and Rac-GAP domain. Initially we tried to solve the structure of this protein by molecular replacement using Rac-GAP domain and C1 domain as search model. Since this method was not successful, we collected MAD data sets of native(Zn^{2+}) and Hg derivative to 4.0 Å and to 4.5 Å, respectively. Now we are trying to solve phase problem out of these data sets.

Second, MAD data sets of DAX domain were collected. DAX domains exist in dishevelled and axin proteins,

molecules in Wnt signaling pathway, and are considered to play roles in their multimerization. We used Br-soaked crystals to apply MAD method, and diffracted at 3.2 Å, which diffracted only to 7.0 Å(Home source) and to 5.0 Å (2nd generation synchrotron). To solve phase problem with this method, generally higher resolution is required. We need further improvement of crystals and data.

Third, we collected data sets of Inositol polyphosphate 5-phosphatase catalytic (IPP5C) domain in complex with IP₃. Since the structures of IPP5C domain in apo form and in complex with IP₂ had already been solved, we collected only the native data set to 2.2-2.4 Å, which diffracted only to 4.2 Å at home source. Information that we get from the structure of IPP5C-IP₃ complex will explain the detail of the catalytic mechanism of the inositol polyphosphate 5-phosphatase.

X-ray crystallography of human DNA homologous recombination protein, Rad52, and its complex with single-stranded DNA

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Human Rad52 (HsRad52) protein is essential for DNA recombination and DNA repair as human Rad51 and *E. coli* RecA proteins. Electron microscopic analysis revealed that seven HsRad52's forms a ring structure to accommodate single-stranded DNA. To elucidate how HsRad52 binds to DNA and accomplishes DNA recombination and DNA repair, we tried co-crystallization of several constructs of DNA-binding domain of HsRad52 and several lengths of single-stranded DNA. The best crystals up to date belong to the space group $P4_32_12$ with unit-cell parameters $a = b = 145 \text{ \AA}$, $c = 243 \text{ \AA}$, and diffracted X-ray beyond 3.2 Å resolution in this beam time. By MAD experiment using SeMet derivative crystals, we determined the initial phase with 7 out of 33 Se sites. Density modification with NCS averaging significantly improved the phase, and the interpretation of the electron density map is now in progress.