Poster Presentation

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Intentional crystal-contact-free space in protein crystal

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To understand the function of proteins, it is essential to perform the structural analysis of the protein complexes with ligands, such as substrates or partner molecules. The motions of ligands are restricted by the contacts with neighbor protein molecules in the crystal lattice. Here, we propose a new technique to analyze dynamics of a ligand in the bound state preserved in the crystal-contact-free space, which is intentionally created in protein crystals. We used Tom20 as a target protein. Tom20 functions as a general protein import receptor, by recognizing N-terminal signal sequences (presequences) of mitochondrial matrix proteins. Our working hypothesis is that the promiscuous specificity of Tom20 is attributed to the large mobility of the presequneces in the binding groove of Tom20 (1,2). Our aim is to obtain electron density that reflects the large mobility of a presequence in the crystal-contact-free space. In order to create the crystal-contact-free space, we took advantage of a protein fused with maltose binding protein (MBP). The key of the design is the connection of the two proteins firmly. We fused the C-terminal α-helix of MBP and the N-terminal α-helix of Tom20 seamlessly. After a systematic model building study, we decided to use a design with four residues inserted in the linker region. We found smeared electron density in the binding site of presequences in the difference Fourier electron-density map. We attached an iodine atom at the N-terminus of the presequence and confirmed the N-terminal position in the smeared electron density. We performed molecular dynamics simulation without the tethering in solution (3). The electron density simulated from the MD trajectory was fully consistent with the smeared electron density in the crystal contact-free space. We concluded that the smeared electron density corresponded to the partially overlapping region of the multiple states of the bound presequence.

[1] *Saitoh et al., EMBO J (2007) 26, 4777–4787,* ***[2]*** *Saitoh et al., Biochemistry (2011) 50, 5487–5496,* ***[3]*** *Komuro et al., J Phys. Chem B (2013) 117, 2864-2871*

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