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The EU BIOXHIT Standard Test Crystal - Verifying Se-MAD Beamlines

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The standard test crystal is designed to provide an automated, fast and robust procedure for identifying potential problems in the complex hardware and software infrastructure of modern protein crystallographic (PX) synchrotron beamlines. The resulting diagnostics should be independent of the data integration software employed (XDS, MOSFLM/SCALA and HKL2000).
The cubic form of bovine insulin (space group $I2_13$) has proved to be a good first choice for a test crystal, with high reproducibility in crystal growth and freezing, high symmetry for highly redundant data collection, there is an additional need to verify the operation of MAD beamlines, in particular those used for phasing of selenomethionine derivatives. For this purpose we have synthesised an organoselenium compound that crystallises in the suitable space group $P4_12_12$ and has four selenium atoms in general positions in the asymmetric unit. We have determined precise cell dimensions at various temperatures by X-ray powder diffraction to test the possibility that the cell can be reproduced well enough to provide a precise wavelength calibration for MAD beamlines. We will present our experiences with the new Se-MAD test crystal.
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Structural Studies of the Nucleosome Remodeling Factor CHRAC

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Nucleosome remodeling factors confer dynamic properties to chromatin and render nucleosomal DNA accessible for interacting factors. The presence of the ATPase ISWI characterizes one subgroup of remodeling complexes, which comprises the remodeling complexes NURF, ACF and CHRAC. We are focusing on the structural analysis of the *Drosophila* chromatin accessibility complex (CHRAC), which consists of the two large subunits ACF1 and ISWI and the two smaller histone-like subunits CHRAC-14 and CHRAC-16.
ISWI belongs to the SWI2/SNF2 subfamily of DEAD/H helicases and contains two SANT related modules at its C-terminal end. We have identified a substrate recognition domain spanning the C-terminal half of the molecule and solved its crystal structure at 1.9 Å resolution [1]. The crystal structure consists of three domains: a four-helix-domain with a novel fold and the two α-helical domains SANT and SLIDE (SANT-like domain), which are linked by a long spacer helix. SANT and SLIDE domains are both structurally related to the DNA-binding modules of the transcription factor c-Myc, but interact differently with nucleosomal substrates.
The crystal structure of the CHRAC-14/CHRAC-16 heterodimer - which distinguishes CHRAC from the related ACF complex - shows a variant histone-fold. Functional analysis shows a facilitating role of the CHRAC-14/CHRAC-16 heterodimer in nucleosome sliding [2]. In order to obtain information about the entire remodeling factor/nucleosome complex we are now assembling the ATPase ISWI with nucleosome substrates. Structural studies using electron microscopy and protein crystallography are combined with biophysical experiments using analytical ultracentrifugation and small angle X-ray scattering.

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