methylated proteins tend to diffract to higher resolution and show lower isotropic temperature factors. A number of well-ordered methylated lysines have been identified. Some lysine residues remain unmethylated or monomethylated although excess of reagents was used. These methylated residues make both inter- and intra-molecular contacts. We describe a detailed protocol, results, success rates and specific interactions in protein crystals that contribute to improved crystallization properties of some proteins. This work was supported by National Institutes of Health Grant GM074942 and by the U.S. Department of Energy, Office of Biological and Environmental Research, under contract DE-AC02-06CH11357.

Keywords: reductive methylation, crystal packing, isotropic temperature factor

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Many are called but few are chosen: 20 years of crystallizing HIV-1 reverse transcriptase

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Our research efforts focus on crystallographic studies of HIV-1 reverse transcriptase (RT), a multifunctional enzyme of the AIDS virus and the target of many of the most widely used anti-AIDS drugs. Over the past 20 years, we have crystallized and solved a diverse array of three-dimensional structures representing distinct conformational states of HIV-1 RT and clinically relevant drug-resistant mutants; these include HIV-1 RT in complexes with inhibitors, nucleic acid substrates, and a monoclonal antibody Fab fragment [1]. Our desire to improve both crystal quality and diffraction resolution led to improvements in purification methods and successful crystal engineering studies by site-directed mutagenesis, leading to 1.8 angstrom resolution diffraction for several crystal forms [2]. Recently, a longstanding collaboration between our lab and Janssen Pharmaceutica/Tibotec, Belgium, culminated in FDA approval of the potent non-nucleoside RT inhibitor etravirine/Intelence/TMC125 for treatment of HIV-1 infections resistant to other antiretroviral agents. Another related non-nucleoside RT inhibitor, TMC278, is more effective against drug-resistant HIV-1 variants than any other compound reported to date, and is currently in Phase III clinical trials [3]. Various methodologies used by our laboratory to produce diffraction-quality crystals of a number of RT complexes are described.
[1] Clark A.D., et al, Methods Enzymol, 1995, 262, 171-85, [2] Bauman J.D., et al, 2008, submitted, [3] Das K., et al, PNAS, 2008, 105, 1466-71.

Keywords: HIV-1 reverse transcriptase, protein purification crystallization, HIV drug design

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Studies on enzymes belonging to the crotonase superfamily

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The crystal structure of delta3-delta2-enoyl-CoA isomerase from human mitochondria (hmEci), complexed with the substrate analogue octanoyl-CoA, has been refined at 1.3 Å resolution. This enzyme takes part in the β-oxidation of unsaturated fatty acids by converting both cis-3 and trans-3-enoyl-CoA esters (with variable length of the acyl group) to trans-2-enoyl-CoA. hmEci belongs to the hydratase/isomerase (crotonase) superfamily. Most of the enzymes belonging to this superfamily are hexamers, but hmEci is shown to be a trimer. The mode of binding of the ligand, octanoyl-CoA, shows that the ω-end of the acyl group binds in a hydrophobic tunnel formed by residues of the loop preceding helix H4 as well as by side chains of the kinked helix H9. From the structure of the complex it can be seen that Glu136 is the only catalytic residue. A cavity analysis shows the presence of two large, adjacent empty hydrophobic cavities near the active site, which are shaped by side chains of helices H1, H2, H3 and H4. The structure comparison of hmEci with structures of other superfamily members, in particular of rat mitochondrial hydratase (crotonase) and yeast peroxisomal enoyl-CoA isomerase highlights the variable mode of binding of the fatty acid moiety in this superfamily.

Keywords: oxyanion hole, coenzyme A, isomerase

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Structure analysis of ligand-independent activation of Fushi tarazu factor-1 ligand binding domain

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Drosophila melanogaster Fushi tarazu factor 1 (Ftz-F1) is an orphan nuclear receptor of which ligand has not been identified until now. The Ftz-F1 regulate gene expression for development, reproduction and cholesterol homeostasis. Also, It is known that the Ftz-F1 interacts with segmentation gene 'Fushi tarazu' (Ftz) for activation of the Ftz-F1. The Ftz-F1 is divided two parts, DNA-binding domain (DBD) and ligand-binding domain(LBD). It is known which ligand binding domain of Ftz-F1 is crucial part to regulate gene expression. Here we report the crystal structure analysis of the Ftz-F1 LBD bound to the peptide containing LXXLL co-activator motif of Ftz. The Ftz-F1 LBD structure consists of eleven helix and two beta strand which form a fourth-layer alpha-helical sandwich. Compared to the structures of Liver receptor holmologue-1 and Steroidogenic factor-1 of the same subfamily of nuclear receptor, the Ftz-F1 LBD does not have enough space for ligand-binding which explains in structural points why the ligand for Ftz-F1 have not been found even though extensive efforts searching for it. Interestingly Ftz-F1 has the AF-2 in the active conformation without ligand binding. These suggest that Ftz-F1 is a constitutively active nuclear receptor which does not need ligand implying the another regulation mechanism of the Ftz-F1