interactions. NMR shows that there is a reduction in the backbone mobility on the regions of the PHD that participate in the peptide binding, and binding affinities differ depending on histone tail lengths. Thermodynamic analysis reveals that the discrimination in favor of methylated lysine is entropy driven, contrary to what has been described for chromodomains. The molecular basis of H3K4me3 recognition by ING4 differs from that of ING2, which is consistent with their different affinities for methylated histone tails. These differences suggest a distinct role in transcriptional regulation for these two ING family members due to the antagonistic effect of the complexes that they recruit onto chromatin. Our results illustrate the versatility of PHD fingers as readers of the histone code.

Keywords: ING, inhibitor of growth, PHD, plant homeodomain, histone 3

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A new type of precipitant, metal cyanide complex

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Transition metal salts, such as Fe, Ni, Co, and Cr salt, are generally avoided in protein crystallization because of fear of protein inactivation or denaturation. However, if transition metal is incorporated in crystal structure, they are useful for SAD, or MAD phasing. Here we cast spotlight on metal cyanide complex salts as protein precipitant, because metal cyanide complex has relatively low affinity for protein and chemically stable over a wide range of pH. We tested five metal cyanide complex salts, $K_3Cr(CN)_6$, $K_3Fe(CN)_6$, $K_4Fe(CN)_6$, $K_3Co(CN)_6$, and $K_2Ni(CN)_4$. Three proteins, lysozyme, proteinase K, and trypsin were tried to crystallize using each salt as a precipitant by batch method. No additional compound added to mother liquid without buffer component (from pH 4 to pH 9). Diffraction data from grown crystals were collected using CuKα radiation. Metal sites were located using anomalous signal by the program *SHELXD*. Phase calculation was performed by SAD method using program *MLPHARE* and automatic model-building by *ARP/wARP* were performed. All tested protein can be crystallized by metal cyanide complexes. The crystal form was isomorphous to that grown with non-metal precipitant except in the case of lysozyme with $K_4Fe(CN)_6$, where we obtained a new crystal form. In the crystal, metal complexes bind to positively-charged surfaces of the protein. In the case of Cr, Fe, and Co salt, location of metal sites, phasing by SAD method, and automatic model-building were accomplished easily and successfully. But, in the case of Ni salt, location of metal sites was difficult, and phasing power on SAD phasing is as low as that of native (non-metal) crystal because of low f" value of Ni at the wavelength of CuKα radiation.

Keywords: new type of precipitant, transition metal complex, SAD method

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Crystallization and crystallographic analysis in a microfluidic chip

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Microfluidic technology opened new possibilities for the crystallization of biological macromolecules. Indeed, microfluidic systems offer a lot of advanges for crystal growth: they enable easy handling of nano-volumes of solutions and, thus, extreme miniaturization and parallelization of crystallization assays. In addition they provide a convection-less environment a priori favorable to the growth of high quality crystals. Pioneer examples implementing free interface diffusion [1] and nano-batch [2] crystallization in microfluidic chips have already demonstrated the value of this technology, especially for high throughput screening applications in structural genomics. We will present the results of a collaborative work initiated in 2004, that is focused on the design of a versatile, low cost and easy-to-use crystallization chip. A novel chip based on the counter-diffusion of solute molecules playing the role of crystallization agents will be described [3]. The chip is made of rigid polymers (e.g. PMMA, COC) that are impermeable to gases and compatible with crystal examination and monitoring in polarized light. Selected materials are also transparent to X-rays, and three-dimensional protein structures can be determined from crystals contained inside this device (image on the left) using X-ray diffraction data collected on a synchrotron source (middle). The outstanding quality of the electron-density maps (right) demonstrates that on-chip crystal analysis is feasible.

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Keywords: biomacromolecule crystallization, microsystems, *in situ* diffraction

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Improving protein crystallization: A large-scale evaluation of protein reductive methylation

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Obtaining diffraction quality crystals is a major bottleneck in protein X-ray crystallography. Chemical modification of proteins can alter their surface properties and crystallization behavior and influence crystallization success. We evaluated how the methylation of lysine residues in proteins may improve crystallization on a set of 370 unique proteins (<30% sequence identity) that failed to produce diffraction quality crystals. Applying well-established methods, the proteins were methylated and screened using standard crystallization procedures. Crystal structures of 26 new proteins were determined, four in both native and methylated states. Crystals of

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.
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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