

In the last years, we have studied a nuclear protein complex formed by the association of histone deacetylase 1, a co-repressor protein CoREST, and lysine-specific histone demethylase LSD1 that specifically acts on Lys4 of histone H3. This LSD1/HDAC/CoREST multi-enzyme module functions as a “double-blade razor” that first eliminates the acetyl groups from acetylated Lys residues and then removes the methyl group from Lys4 [1], [2]. Our structural studies of the CoREST/LSD1 complex highlighted a uniquely specific binding-site for the histone H3 N-terminal tail and a catalytic machinery that is closely related to that of other flavin-dependent amine oxidases. These insights have been critical for our efforts towards structure-based development of demethylase inhibitors. These newly designed inhibitors were evaluated with a cellular model of acute promyelocytic leukemia chosen since its pathogenesis includes aberrant activities of several chromatin modifiers. Marked effects on cell differentiation and an unprecedented synergistic activity with anti-leukemia drugs were observed [3].

It has been recently discovered that the transcription factor Snail1 binds to LSD1/CoREST and that the three proteins are over-expressed in cancer cell lines and breast tumors. Snail1 controls the epithelial-mesenchymal transition, which is essential for numerous developmental processes (including metastasis). Structure determination of the ternary complex LSD1/CoREST/Snail1 peptide has revealed that Snail1 interacts with LSD1/CoREST in a remarkable way: the N-terminal residues of this transcription factor bind in the active site cleft of LSD1 effectively mimicking the histone H3 tail. Therefore, Snail1 is a potential endogenous inhibitor of LSD1. Furthermore, this finding predicts that other members of the Snail1-related transcription factor family associate to LSD1/2 through a similar histone-mimicking mechanism [4].

A challenge for future studies will be to extend these structural investigations to visualize nucleosome binding by LSD1-containing protein complexes through biophysical methods and crystallography.

Supported by AIRC and Fondazione Cariplo

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**Key-words: chromatin, transcription, protein\_complex**

## MS.15.4

*Acta Cryst.* (2011) A67, C50

### Structural basis of the versatile DNA recognition by the methyl CpG binding domain of MBD4

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DNA methylation is one of the major epigenetic marks associated with a repressed chromatin state and gene silencing, and is involved in regulation of various physiological events such as X chromosome inactivation, embryogenesis and genomic imprinting. Cytosine methylation at the C5 position in CpG (5'-CG-3') dinucleotides is the only covalent modification in mammalian genomic DNA. The DNA methylation pattern established during developmental processes determines characteristics of each cell type. Various multi-protein complexes are recruited on the methylated CpG sites and regulate gene expression. Methyl-CpG binding domain (MBD) proteins bind to methylated CpG sites and recruit other protein factors including histone

modification enzymes and chromatin remodelling factors, generally resulting in establishment of silent chromatin.

MBD4 is a unique MBD family member that contains a glycosylation domain, and plays an important role in DNA repair as a thymine glycosylase to remove T/G mismatches generated after the deamination of 5-methylated cytosine (<sup>5m</sup>C). Recently, MBD4 has been shown to be involved in active DNA demethylation in mammalian cells. The active demethylation has been to date suggested to be mediated at least partly by a base excision repair pathway involving converting of <sup>5m</sup>C to thymine by the AID/Apobec family of deaminases followed by G/T mismatch repair by the DNA glycosylase MBD4 [1]. Furthermore, MBD4 has been implicated to directly facilitate demethylation by its glycosylation activity towards the <sup>5m</sup>C base depending on its phosphorylation state [2]. Towards full understanding of the molecular mechanism underlying DNA demethylation, we characterized the DNA binding specificity of the MBD domain of mouse MBD4 (MBD<sub>MBD4</sub>) and determined its crystal structure in the complex with a DNA fragment containing symmetrically methylated CpG (<sup>5m</sup>CG/<sup>5m</sup>CG) or its deamination product (<sup>5m</sup>CG/TG).

Combined with biochemical data, our structures showed MBD<sub>MBD4</sub> binds to both of symmetrically methylated <sup>5m</sup>CG/<sup>5m</sup>CG and <sup>5m</sup>CG/TG mismatch sequences in B-form DNA duplex with similar affinities. MBD4 shares an essential recognition mode for <sup>5m</sup>CG/<sup>5m</sup>CG with structurally known MBD domains of MBD1 [3] and MeCP2 [4]. However, the DNA binding interface of MBD<sub>MBD4</sub> is modulated to enable bi-substrate recognition of <sup>5m</sup>CG/<sup>5m</sup>CG and <sup>5m</sup>CG/TG. There are two key structural features for the versatile DNA recognition: flexibility of the arginine finger responsible for base recognition and the DNA binding pocket containing a water-molecule network. Moreover, our biochemical data have demonstrated the inhibitory effect of MBD<sub>MBD4</sub> in the T/G mismatch glycosylation activity of its isolated glycosylase domain. This result implies unknown molecular mechanism for the substrate hand over from MBD<sub>MBD4</sub> to the glycosylase domain in the context of full-length protein.

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**Keywords: biomolecule, DNA, protein**

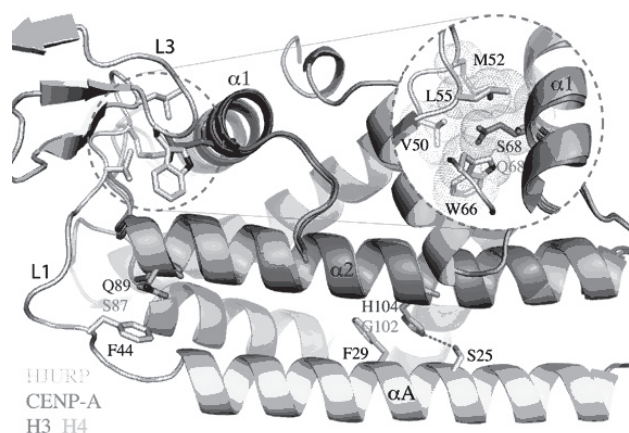
## MS.15.5

*Acta Cryst.* (2011) A67, C50-C51

### Structure of a CENP-A-histone H4 heterodimer in complex with chaperone HJURP

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The centromere is epigenetically specified by the histoneH3 variant Centromere Protein-A (CENP-A). In higher eukaryotes, deposition of CENP-A to the centromere requires histone chaperone HJURP (Holliday junction recognition protein). The crystal structure of an HJURP-CENP-A-histone H4 complex shows that HJURP binds a CENP-A-H4 heterodimer. The C-terminal  $\beta$ -sheet domain of HJURP caps the DNA-binding region of the histone heterodimer, preventing it from spontaneous association with DNA. Our analysis also revealed a novel site in CENP-A that distinguishes it from histone H3 in its ability to bind HJURP. These findings provide key information for specific recognition of CENP-A and mechanistic insights into the process of centromeric chromatin assembly.



Structure of human CENP-A-H4-HJURP Complex

**Keywords:** centromere, CENP-A, histone chaperone

### MS.16.1

*Acta Cryst.* (2011) A67, C51

#### Engineering immunity against HIV-1 using designed antibody constructs

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Despite decades of effort, no current vaccine elicits neutralizing antibodies at concentrations blocking HIV-1 infection. In addition to structural features of HIV's envelope spike that facilitate antibody evasion, we proposed that the low density and limited lateral mobility of HIV spikes impedes bivalent binding by antibodies via inter-spike cross-linking [1]. In addition, molecular modeling suggested that bivalent binding within a single trimeric spike (intra-spike cross-linking) is also unlikely for antibodies directed against most protein epitopes. The resulting predominantly monovalent binding minimizes avidity and thereby high affinity binding and potent neutralization, thus expanding the range of HIV mutations permitting antibody evasion. In this talk, I will review our efforts to create high avidity anti-HIV protein reagents for use in gene therapy and/or passive immunization. One class of reagents is based upon a naturally-occurring dimeric form of 2G12, a neutralizing antibody that recognizes carbohydrates on the gp120 portion of the HIV spike. 2G12 monomers use both Fabs in an unusual domain-swapped (Fab)<sub>2</sub> unit to recognize a constellation of carbohydrates on gp120. We have shown that dimerization of 2G12 leads to enhanced potency against HIV-1 strains that are sensitive to 2G12 monomers and neutralization of strains that are resistant to 2G12 monomers [2]. Thus carbohydrate-binding reagents are a logical starting point for engineering novel bivalent and multivalent antibody architectures capable of intra-spike cross-linking. Another class of engineered reagents we're working on involves fusion of the first two domains of the host receptor CD4 to the variable regions of an antibody recognizing the CD4-induced (CD4i) co-receptor binding site on gp120. We designed, expressed, purified, and tested the neutralization potencies of CD4-CD4i antibody reagents with different architectures, antibody combining sites, and linkers [3]. Implications of a crystal structure of a clade C gp120/CD4/CD4i Fab complex that demonstrates auto-reactive binding between the CD4i antibody and CD4 [4] will also be discussed.

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**Keywords:** antibodies, HIV-1, gp120

### MS.16.2

*Acta Cryst.* (2011) A67, C51

#### The multiple personalities of transthyretin

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The human plasma protein transthyretin (TTR) is a soluble protein that functions as transport protein for thyroxine. At certain conditions however the normally tetrameric protein dissociates and forms structurally less defined monomeric or dimeric species that are prone to aggregate and form fibrils/amyloids leading to disease — familial amyloidotic polyneuropathy (FAP, type I). One of our aims is to characterize in detail the structural changes in the TTR protein that lead to amyloid formation and disease [1], [2].

To prevent transthyretin fibril formation, one rather successful approach is to stabilize the native state structure, thereby reducing the protein's ability to form the misfolded intermediate structures needed to form fibrils [3]. Even though a number of stabilizing compounds have been found [4], [5], it is still desirable to find new and more structurally diverse scaffolds, and for those reasons we have initiated a fragment-based lead generation campaign [6] using human transthyretin as target protein. In this presentation, we will review our experiences and some of the results observed.

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**Keywords:** folding, drug design, stability

### MS.16.3

*Acta Cryst.* (2011) A67, C51-C52

#### Structural biology and medicinal chemistry in neglected diseases of poverty

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Parasitic diseases are a major global cause of illness, morbidity, long-term disability, and death, with severe medical and psychological