A wide range of minor groove binding ligands (MGBLs) with good sequence discrimination ability are of interest as potential therapeutic agents in variety of human diseases such as cancer, along with anti-bacterial and/or anti-parasitic activities. These MGBLs are highly selective to A:T base pairs of the minor groove of the DNA. Some of these compounds are in phase III clinical trials while some are currently in use for their veterinary applications.

Nucleic acids require structured water molecules in order to maintain their stability, polymorphism and flexibility of the duplex DNA. The binding of ligands into the minor groove of DNA involves the displacement of the native (drug-free) structured water molecules. These ligands have been studied extensively over the last two decades using a number of methods. However, for many of these systems understanding regarding water interactions and protonation states of the DNA-ligand complex and components remains unclear. This critical information is important for understanding the stability and recognition of DNA ligand complexes. Crystallographic methods have been used to determine the molecular structure of small molecules bound to DNA sequences, in order to better understand the details of molecular recognition by DNA.

Some of the MGBLs have shown to be effective inhibitors of a number of minor and major groove binding protein-DNA interactions (e.g. OTF 1, Atpy HD, HMGA2, etc.) [2]. Studies have been done with major groove binding transcription factor NF-kB which will add additional detail towards the biological significance and activity for the MGBLs. In this project we aim to build a library of information relating drug-DNA-water interactions to sequence specificity and drug design using X-ray crystallography as well as kinetics (e.g. from SPR) data and gel shift assays. This information is valuable for rational drug design in future.

Keywords: crystallography, DNA, drug

MS16.P02


**Specificity and efficiency in activity of anti-HIV actinohivin for sugar binding**
Md. Mominul Hoque,*,**, Jiandong Jiang,† Kaoru Suzuki,‡ Masaru Tsunoda,§ Atsushi Takahashi,∥ Takeshi Sekiguchi,∥ Haruo Tanaka,∥ and Akio Tsukana*∥∥*Faculty of Pharmacy, †Graduate School of Science and Engineering, ‡Faculty of Science and Engineering, §Iwaki-Meisei University, ¶Iwaki 970-8551, (Japan), ‡Dept. of Biochemistry & Mol. Biol., University of Rajshahi, Rajshahi, Bangladesh ∥Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Yokohama 226-8501, (Japan). E-mail: mominbio@yahoo.com

In order to overcome the present multi-drug resistance problem in treating HIV/AIDS pandemic, a new lectin actinohivin (AH) was found to exhibit a potent anti-HIV activity through binding the higmannose type glycans (HMTG) which are bound to gp120 of HIV. X-ray analyses of AH and its complex with o-(1,2)-mannonobise (M2) which is the terminal end of the three branches (D1, D2 and D3) of HMTG revealed that AH is composed of tandem repeated three structural modules associated with a pseudo three-fold symmetry[1]. In each module, M2 is accommodated through the specific hydrogen bonds with D, Y and N residues equivalent between the three modules.

In this study, the structural features were revised, as seen in Fig. 1(a), based on which essential residues for specific inter-actions were confirmed by mutation experiment[2]. In addition, dimerization effect of AH on anti-HIV activity was examined by increasing the number of HMTG-binding sites in a molecule, because gp120 is covered with many HMTGs, as seen in Fig. 1(b). Several dimeric AH (AH2) derivatives were prepared and their anti-syncytium formation and anti-HIV activities were evaluated.

D15, Y23, L25, N28 and Y32 (in module I) and the corresp-ponding residues in the other modules were identified to be essential for AH activity. Among them, D, Y and N residues participate in recognition of M2. By superimposing the three terminal ends of D1 branches onto the bound M2, a model of gp120 bound to several AHs has been constructed. This model suggests that the AH affinity to gp120 is amplified in col-laborative binding when it forms a dimer as a cluster. Among several AHs (see Fig. 1(c)), those linked by a head-to-tail fashion showed higher (20 folds at maximum) in anti-syncytium formation activity and (2–30 folds) in anti-HIV activity than those of AH monomer. These activities vary depending on the linker sequence. Therefore, the anti-HIV activity of AH can be improved as a microbicide to prevent HIV transmission.

Keywords: crystallography, DNA, drug

**Fig. 1. Three M2s bound in the three modules of AH (a), a model of several AHs bound to gp120 (b), and an image of designed AH2 associated to each other through dimerization domains (c).**


MS16.P03


**Crystal structures of DNA containing X relevant to gastrointestinal cancer**
Fang Zhang,*,‡ Jiandong Jiang,† Kaoru Suzuki,‡ Md. Mominul Hoque,*, Masaru Tsunoda,§ Oliver Wilkinson,*,‡ Christopher L. Millington,‡ David M. Williams,‡ Akio Takéнакa,∥∥∥∥Graduate School of Science and Engineering, †Faculty of Science and Engineering, §Iwaki-Meisei University, ¶Iwaki 970-8551, (Japan), ∥Center for Chemical Biology, Krebs Institute, University of Sheffield, Sheffield S3 7HF, (UK), ‡Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Yokohama 226-8501, (Japan). E-mail: zhangfanguknow@hotmail.com

Red meat stimulates endogenous intestinal N-nitrosation of glycine and its derivatives that can induce DNA mutations by reacting with DNA to form O6-methylguanine (hereafter X) which is associated with increased risk of gastrointestinal cancer. In order to obtain insights into the pairing geometry of DNA duplexes containing X (modified or damaged nucleotide) and to further understand its biological implications, we have determined the crystal structures of two DNA dodecamers with the sequences d(CGCGAATTCGCG) (hereafter X:C) and d(CGCGAATTCGCG) (hereafter X:T) by X-ray analyses.
In both crystals of X:C and X:T, two dodecamers are associated to form a right-handed double helix with B-form conformation similar to those of the unmodified duplexes. The carboxymethyl groups of the damaged nucleotides are protruded into the major groove of the duplex suggesting that carboxy-methylation has no significant effects on the DNA conformation. In the X:C duplex, X and its complementary C base form a pair to each other through the two hydrogen bonds, N(X)…N(C) and N(X)…C(N), so that the two bases are displaced in different directions, X toward the major groove side and C toward the minor groove side. The wobbling directions are reversed from those found frequently in G:T pairs. In addition, this pairing is further stabilized by an additional hydrogen bond between the carboxyl oxygen and the paired C amino group. In the X:T duplex, however, the X residue forms a pair with T in a Watson-Crick type geometry with propeller twisting to release the O…O interaction. A model building of DNA polymerase in complex with X-containing DNA has suggested that these pairing modes found in the present study are acceptable for incorporation of dCTP or dTTP opposite X by the polymerase. It implies that the X residues modified in a DNA template not only direct the incorporation of complementary dCTP but also allow the non-complementary dTTP to be incorporated into the newly synthesized DNA strand. Therefore, it can be concluded that the latter Watson-Crick type X:T pair formation induces GC-to-AT transition mutation of genes demonstrated by \textit{in vitro} and \textit{in vivo} experiments, as an origin of increased risk of gastrointestinal cancer.

Fig. 1. Two pairing modes found in X:C(a) and X:T(b)

**Keywords:** crystal structure, damaged DNA, mutagenesis

### MS16.P04

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**Crystal structure of the human thioredoxin reductase - thioredoxin complex implicates movement of the C-terminal redox center by 20 Å upon catalysis**

Karin Fritz-Wolf, Sebastian Kehr, Michaela Stumpf, Stefan Rahlfis, Katja Becker

Interdisciplinary Research Centre, Justus Liebig University, D-35392 Giessen, (Germany). Max Planck Institute for Medical Research, D-69120 Heidelberg, (Germany). E-mail: karin.fritz-wolf@mpimf-heidelberg.mpg.de

We present the first crystal structure of human TrxR1 (hTrxR) in complex with its substrate thioredoxin (Trx). The human thioredoxin system comprises the homodimeric flavoenzyme thioredoxin reductase, its characteristic substrate thioredoxin and NADPH. TrxR is crucially involved in the regulation of cellular redox reactions, growth and differentiation. Its importance in various diseases renders TrxR1 a highly interesting drug target. During catalysis electrons are transferred from the cofactor NADPH to FAD, then to the N-terminal active site cysteines and from there to the C-terminal active site to be finally delivered to Trx at the molecule’s surface. We used X-ray crystallography to depict crucial conformations of the TrxR carboxyterminal amino acid chain at different stages in the catalytic cycle of the enzyme. In the complex structure the C-terminal redox center is found about 20 Å apart from the N-terminal redox center. Our structure and the analysis of hTrxR1 mutants shows that the enzyme recruits a swinging C-terminal arm for electron transport to its substrates, which is stabilized by a guiding bar for controlled transfer. Furthermore, essential residues of the interface region were characterized both structurally and functionally.

The three-dimensional structure of the hTrxR1-hTrx1 complex provides an important template for future drug design, and contributes to our understanding of redox regulatory processes in eukaryotes.

**Keywords:** complex, mobility, redox biology

### MS16.P05

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**Ligand Efficiency Indices as a navigation tool in the transferrin protein chemico-biological space to find new amyloidogenic inhibitors**

Daniel Blasi, Gemma Arsequell, Gregory Valencia, Joan Nieto, Antoni Planas, Marta Pinto, Nuria B. Centeno, Cele Abad-Zapatero, Jordi Quintana, ‘Drug Discovery Platform (PDD), Parc Científic de Barcelona, Barcelona (Spain).’ Unit of Glycoconjugate Chemistry, Institut de Química Avançada de Catalunya, IQ-A.C.-C.S.I.C., Barcelona (Spain).’ Laboratory of Biochemistry, Institut Químic de Sarrià, Universitat Ramon Llull, Barcelona (Spain).’ Computer-Assisted Drug Design Laboratory, Research Group on Biomedical Informatics (GRIB) IMIM-Universitat Pompeu Fabra, Barcelona (Spain).’ Center for Pharmaceutical Biotechnology, University of Illinois at Chicago, 900 So. Ashland St, MRBB Building, Room 3020, (MC870), Chicago, IL 60607-7173, (USA). E-mail: dbblasip@pcb.ub.es

We have previously reported the design and synthesis of ligands that stabilize Transferrin protein (TTR) [1] in order to obtain therapeutically active compounds for Familial Amyloid Polyneuropathy (FAP). We are hereby reporting a drug design strategy to optimize these ligands to target Familial Amyloid Cardiomyopathy (FAC). The strategy comprises: i) a SAR analyses of the ligands described previously for the TTR tetramer, classified in chemical classes and mapping them in Chemico-Biological Space (CBS) using Ligand Efficiency Indices (LEIs) [2]; ii) optimization through docking in the TTR tetramer three-dimensional structure model; and iii) improvement of physicochemical/pharmacokinetic/selectivity properties.

We have focused in the optimization of TTR amyloid inhibitors exploring new methods to guide the proposed drug discovery strategy. Our approach is based on using Ligand Efficiency Indices (LEIs) [2, 3, 4] to navigate and evaluate the efficiency of the existing TTR ligands reported on the Protein Data Bank, combining a binding efficiency index (BEI) [4], based on the measured binding affinity related to the Molecular Weight (MW) of each compound, with a surface efficiency index (SEI) based on Polar Surface Area (PSA). We will illustrate the use of these indices, combining three crucial variables (potency, MW and PSA) in a 2D graphical representation, to perform a retrospective mapping of SAR data for a current TTR inhibitors database, taking as starting point of the analysis a good crystallographic ligand (iododiflunisal). We will also demonstrate how LEIs can be used for prospective strategies of optimization and drug design efforts for new TTR inhibitors [5].