## MS.77.3

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**Revisit the basics to optimize detection systems and time-resolved XAFS**

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XAFS has become one of the most important research tools. And it has become easier to take high quality XAFS spectra with the development of many synchrotron facilities. Top-up injection made the synchrotron beam more stable then before.

Needless to say, linear response of the detectors for incoming signal is very important to obtain reliable results. The continuity of XAFS spectra before and after an injection was a good index to check the linearity of the detection systems and stability of the beamline optics. However, it is overlooked by some experimenters. And it is getting difficult to check them with the development of top-up injection. Ionization chamber is one of the most important detectors to measure XAFS. Linearity of ionization chamber can be realized by optimizing the filled gas and electric field. Germanium detector is also a powerful tool to measure fluorescent XAFS of dilute samples. In this case proper deadtime correction [1] is essential. EXAFS signal becomes continuous before and after an injection when the conditions are optimized.

Quick XAFS and dispersive XAFS are two key techniques to measure time-resolved XAFS. Time-resolution of DXAFS is typically milliseconds but it has reached to 100ps by using single-bunch detection. Time required to obtain a quick XAFS spectrum is also becoming milliseconds with the development of some quick scanning techniques. The apparent time-resolutions seem similar but we have to remind that a spectrum is measured simultaneously thus is a time averaged spectrum in the case of DXAFS. But the composition changes during a measurement of a spectrum in case of QXAFS. A simulation will be demonstrated.

[1] M. Nomura, *J. Synchrotron Rad*. **1998**, 5, 851.

**Keywords: DXAFS, quick XAFS, detector**

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**Development and adoption of scientific data exchange frameworks: a CIF perspective**

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In developing a data exchange specification, the XAS community is treading a similar path to that trodden by the developers of the Crystallographic Information Framework (CIF) 20 years ago. Development of the XAS standard specification will benefit from understanding the reasons for the success of CIF in small-molecule crystallography, and conversely the reasons for the less widespread adoption of CIF in other crystallographic fields.

CIF consists of a simple syntax coupled with domain-specific dictionaries developed by experts in the respective fields. The original highly successful CIF effort produced both the syntax and a "core dictionary" for small-molecule crystallography. This core was supported by the IUCr journals, prominent databases, and major software providers. Authors were initially offered faster publication in IUCr journals when presenting publication-ready results in CIF form, and tools were provided that automatically checked CIF files and transformed them into publication-ready PDF files.

Other crystallographic domains (for example powder and macromolecular crystallography) have generally seen much slower adoption of CIF as an exchange format. Reasons appear to include (a) inability to update and/or redistribute legacy software; (b) CIF provides no benefit compared to current practices; (c) lack of broad community support.

The XAS data exchange development process should therefore ensure (a) that widely-used software packages will support the format; (b) that the XAS community is in general agreement with both the goals and the XAS-specific descriptions contained in the specification; and (c) that there is some benefit to be gained from using the new format. Such a benefit could be created by developing and encouraging minimum standards for presentation of XAS data, while providing software that checked conformance to these standards and leveraged the extra metadata to provide new functionality.

**Keywords: CIF, XAS, data exchange**

## MS.77.5

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**Element selective X-ray imaging of growing chemical patterns**

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Chemical pattern formation has been extensively studied because of interesting similarity to patterns on biological systems. Chemical reaction-diffusion systems generates spatial variations in the concentration fields of the reacting chemicals. In the present research, time-spatial distribution of elements have been studied by element selective X-ray imaging using high-flux synchrotron radiation. Though conventional scanning-type X-ray imaging with micro/nano beam has been widely used, the technique requires a long measuring time. To see elements in growing chemical patterns, we have employed novel projection-type X-ray imaging without performing any scans. Some X-ray movies of Tarube's aritifical cell and ossillating reactions will be presented.

[1] M. Traube, *Arch. Anat. Physiol. u. wiss. Med.* **1867,** 87-129. [2] K. Sakurai, H. Eba, *Anal. Chem.* **2003,** *75*, 355-359.

**Keywords: non-linear system, real time imaging, X-ray florescence**

## MS.78.1

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**Structural biology of eukaryotic gene transcription**

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RNA polymerases are the central multiprotein enzymes that catalyze DNA transcription and RNA synthesis in all cells. Our laboratory uses a combination of X-ray crystallography and electron microscopy to determine the three-dimensional structures of complexes of eukaryotic RNA polymerases with accessory protein factors and nucleic acid substrates. These structures are complemented by functional studies in vitro and in vivo, to obtain mechanistic insights. We have also stated to investigate the regulation of gene transcription on a cellular level, by measuring RNA synthesis rates genome-wide.

In my talk I will first summarize our current understanding of the structural basis of gene transcription. I will then concentrate on the

most recent studies and unpublished data. In particular, I will present our current model for transcription initiation at a gene promoter [1], the most recent insights into transcription elongation, in particular how RNA polymerase II can arrest and then be reactivated [2], and how the only universally conserved transcription factor, Spt5 (or NusG in bacteria), prevents premature termination by enclosing the DNA and rendering transcription processive [3].

[1] D. Kostrewa et al., P. Cramer *Nature,* **2009**, *462*, 323. [2] A.C.M. Cheung, P. Cramer *Nature* **2011**. [3] F.W. Martinez-Rucobo et al., P. Cramer *EMBO J.* **2011,** *8 March*.

**Keywords: gene transcription, RNA polymearse, multiprotein complex**

## MS.78.2

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**Crystal structures of bacterial and yeast ribosomes**

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The crystal structure of the yeast 80S ribosome determined at 4.15 Å resolution reveals the higher complexity of eukaryotic ribosomes, which are 40% larger than their bacterial counterparts [1]. Our crystals capture the ribosome in the ratcheted state which is essential for translocation of mRNA and tRNA and where the small ribosomal subunit has rotated with respect to the large subunit. We describe the conformational changes in both ribosomal subunits that are involved in ratcheting, and their implications to mRNA and tRNA translocation. Structural rearrangements of the ribosome in the tRNA binding step have been studied on bacterial ribosome model. Discrimination of tRNA on the ribosome occurs in two consecutive steps: initial selection and proofreading. We propose a proofreading mechanism based on comparison of crystal structures of the 70S ribosome with an empty A site or the A site occupied by cognate or non-cognate tRNA [2]. We have shown involvement of tales of ribosomal proteins in stabilization of correct tRNA on the ribosome. We suggest that proofreading begins with stabilization of tRNA anticodon loop with involvement of magnesium ions, following by stabilization of elbow region and accommodation of the acceptor end in the peptidyl transferase center.

[1] 1. Ben-Shem, A., Jenner, L., Yusupova, G., Yusupov, M. *Science,* **2010**, 330, 1203-1209. [2] Jenner L, Demeshkina N, Yusupova G , Yusupov M. *Nat. Struct. Mol. Biol.* **2010**, 17, 1072-1078.

**Keywords: yeast ribosome structure, x-ray, proofreading**

## MS.78.3

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**The *GAL* regulon in *S. CEREVISIAE*: the Gal3p/Gal80p interaction**

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*S. cerevisiae* responds to galactose by activating the coordinated transcription of a family of genes known as the *GAL* genes. This transcription is regulated by the *GAL* regulatory switch, which consists of three key protein components: Gal4p, the transcriptional activator; Gal80p, the repressor; and Gal3p, the transducer. The interplay between Gal3p, Gal80p and Gal4p determines the on/off status of the switch. Gal4p binds the upstream activating sequences of *GAL* genes ($UAS_{gal}$) through its N-terminal domain and activates transcription through interaction of its C-terminal transcription activation domain (TAD) with the transcription machinery. During non-inducing conditions, Gal80p binds to the TAD of Gal4p to repress transcription. Repression is relieved upon induction with galactose, mediated by the transducer, Gal3p. We have shown previously that NADP also plays a role in activating the switch [1]. Gal3p forms a complex with Gal80p, $\alpha$-D-galactose and ATP.

We have solved the crystal structure of the tertiary complex of Gal80p-Gal3p with $\alpha$-D-galactose and ATP to 2.1Å resolution. The structure shows that the interaction between Gal3p and Gal80p occurs only when Gal3p is in a "closed conformation" in which the N- and C- terminal domains of the protein are closer together. This conformation is induced by $\alpha$-D-galactose and ATP binding. The Gal3p interaction surface of Gal80p is different from the surface for Gal4p TAD interaction and the structure reveals the basis for super-repressor phenotypes of Gal80p. Gal3p is 75% identical in sequence to Gal1p, but lacks the galactokinase activity of the Gal1p enzyme. We show that the $\alpha$-D-galactose conformation found in our structure is unique compared to what is found in other galactokinase structures and might be the basis for the lack of galactokinase activity in Gal3p.

[1] Kumar, Rajesh P., *et al. , Science* **2008,** *319,* 1090-1092.

**Keywords: eukaryotic transcription, GAL regulon**

## MS.78.4

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**Structure and function of human BMAL1-CLOCK-DNA complex**

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The molecular mechanism of circadian rhythm from flies to human is evolutionarily conserved as an autonomic oscillator with a transcription activation - inhibition cycle of approximately 24 hours. In human, the circadian transcription control process is regulated by two basic helix-loop-helix (bHLH) PAS domain containing transcription factors, namely BMAL1 and CLOCK as the master controller. The BMAL1/CLOCK complex is proved biochemically to form heterodimers to bind the E-box DNA sequence CACGTG to accomplish their functions.

We present here the crystal structure of BMAL1/CLOCK bHLH domains in complex with DNA at 2.4 Å resolution. The current refined $R_{work}$ and $R_{free}$ values are 24.5% and 29.6%, respectively. The basic regions helices of BMAL1 and CLOCK insert into the major groove of DNA, with the H77, E81, R85 in the BMAL1 and R39, E43 and R37 in the CLOCK as the recognition residues to the E-box sequence CACGTG. The major interactions between these residues and base pairs are hydrogen bonds. The helix after the basic region and the helix after the loop region of BMAL1 and CLOCK form a left handed helices bundle, which contribute to the interaction and recognition region of heterodimer. The heterodimer interface is mainly stabilized by two layers of hydrophobic interactions. Each layer contains four residues, forming the knobs into holes packing. If a BMAL1-BMAL1 homodimer or a CLOCK-CLOCK homodimer model was generated, a series of side chain clashes would be observed. In addition, we observed a dimer of heterodimer in the crystal packing, with the interface between two heterodimers fully mediated by BMAL1. This tetramer form was also confirmed by biochemical and biophysical means with strong functional implications. The two DNA chains in the tetramer