Microsymposia

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AB toxins are important virulence factors for several major bacterial pathogens including Bordetella pertussis, Vibrio cholerae, Shigella dysenteriae and at least two distinct pathotypes of Escherichia coli [1]. They are responsible for massive global morbidity and mortality, accounting for millions of deaths each year, particularly amongst children in developing countries. These toxins are so termed because they comprise a catalytic A-subunit (ADP-ribosylase or RNA N-glycosidase or subtilase activity) that is responsible for toxicity to the host cell, and that is non-covalently linked to a panteneric B-subunit that binds to glycans of the host cell [2]. We separately determined the crystal structures of the A- and B-subunits of a novel AB₅ toxin (SubAB) secreted by Shiga toxigenic Escherichia coli (STEC) to 1.8 Å [3] and 2.08 Å [4], respectively. The study revealed that the A-subunit of this toxin exhibited not only a novel catalytic activity (Subtilase) by targeting the ER chaperone BiP but also showed the first example of a bacterial toxin presenting a marked preference for a non-human synthesised Neu5Gc-containing glycans. We also structurally investigated further the glycan specificity of the B-subunit and these findings will be discussed. More recently, the X-ray structure of the SubAB holotoxin was determined to 2.6 Å. Alanine side directed mutagenesis of the B-subunit amino acid residues that are structurally contacting residues in the A-subunit was performed in order to obtain structural insights into the assembly of such unique architecture. These latest results will be discussed.


Keywords: AB₅, toxin, glycans

MS.36.5

Toxicity and neutralization mechanism of the mqsR:A toxin: antitoxin module
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One mechanism by which bacteria survive environmental stress is through the formation of bacterial persister cells, a subpopulation of genetically identical quiescent cells that exhibit multidrug tolerance and are highly enriched in bacterial toxins. Persister cells are also capable of regenerating a bacterial population after removal of antibiotic, which poses considerable therapeutic challenges given that biofilms are present in over 85% of bacterial infections. Recently, it has been shown that a class of proteins, known as toxin:antitoxin (TA) modules, is highly upregulated in persister cells. Under normal conditions, the toxin and antitoxin associate to form a tight, non-toxic complex. However, under conditions of stress, the antitoxins are readily degraded and the action of the toxins leads to rapid cell growth arrest. The most highly upregulated gene in Escherichia coli persister cells is the bacterial toxin mqsR [1].

Here we used genetic, biochemical and structural studies to show that MqsR, along with MqsA, are a bona fide TA pair that define an entirely novel family of TA modules. The crystal structures (MqsA antitoxin alone, 2.15 Å; the MqsR:MqsA-N complex, 2.0 Å and the MqsA:DNA complex, 2.1 Å [2, 3]) show that the MqsR toxin is a ribonuclease from the RelE bacterial toxin family. MqsR is unique because it is the first toxin linked to biofilms and quorum sensing and is the first toxin that, when deleted, decreases persister cell formation. The antitoxin MqsA is even more atypical because it is the first antitoxin that requires a metal, zinc, for structural stability; it is the only E. coli antitoxin that is structured throughout its entire sequence; and it is the first antitoxin demonstrated to bind DNA via its C-terminal and not N-terminal domain. Critically, MqsA and the MqsR:MqsA complex are also the only known antitoxin/TA pair that bind not only their own promoter but also the promoters of other genes that play important roles in E. coli physiology, including mcrR, spa, espD and the master regulator of stress, rpoS [4]. In addition, our structures also show that upon DNA binding, the MqsA N-terminal domains rotate more than 100° to ‘clamp’ the bound DNA. The binding interaction also induces a 55° curvature of the DNA duplex. Using EMSA and NMR titration experiments, we show that MqsA uses a mechanism of direct readout, mediated by residues Asn97 and Arg101, for DNA recognition. Finally, our most recent work is providing novel insights into the mechanism of MqsR-mediated mRNA cleavage. Collectively, these studies provide the first insights into understanding how the unique MqsR:MqsA TA system mediates the persister phenotype at a molecular level, and, by extension, the multidrug tolerance of E. coli biofilms, which are now being used to develop novel antibacterial therapies that target TA pairs.


Keywords: toxin, transcription, DNA

MS.37.1


Potential impact of an X-FEL on time-resolved studies of protein dynamics

Structural biology is a very successful sub-field of the life-sciences. Technical innovations, including constant improvements surrounding the use of synchrotron radiation, have contributed to an extended acceleration in the rate at which new structures are determined.

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Nevertheless, all enzymes undergo conformational changes during their reaction cycles and an X-ray structure of a resting conformation alone describes only the starting point for the reaction. Time-resolved structural studies of protein reaction dynamics aim to elucidate the conformational changes occurring in proteins and thereby elucidate the chemical details of their reaction mechanism.

First I will describe structural results from time-resolved Laue diffraction studies of a photosynthetic reaction centre performed using synchrotron radiation. In this work we were able to observe conformational changes for a conserved tyrosine residue located near the reaction centre’s special pair of chlorophyll molecules [1], which is photo-oxidised by light. Thereafter I will touch upon the implications of new approaches to time-resolved structural biology which can emerge from the revolutionary new approach of ultrafast nano-crystallography at X-ray free electron lasers [2]. I will describe both the potential benefits of single-shot time-resolved studies from micro-crystals at an X-ray free electron laser, and I will outline some of the challenges associated with time-resolved diffraction using larger crystals at these sources.


Keywords: structural dynamics; jaue diffraction; X-ray free electron laser

MS.37.2


3D imaging with coherent X-rays at nano-scale resolution and beyond
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Progress on coherent X-ray diffractive imaging technique has reached to its successful applications in unveiling three-dimensional structures of biological specimens at several tens of nanometer resolution and nano-structured materials at ten nanometers scale presently. Interest in achieving a few nm resolution is zealous. By overcoming difficulties accompanied by insufficient coherent X-ray flux and X-ray radiation damage to specimens, we expect 3D imaging of a biological cell or organelle at a few nm resolution to be realized in a near future. Further with X-ray free electron laser (XFEL), 3D imaging of small cell or organelle at a few nm resolution to be realized in a near future.

In this talk, I will introduce recent progress on 3D imaging of a yeast cell and others accomplished at SPRing-8. Activities on imaging of biological specimens and nano-structures by using the prototype EUV FEL facility of SPRing-8 Compact SASE Source will also be introduced.


Keywords: coherent X-ray imaging, bio-imaging, free electron laser

MS.37.3


Structure and dynamics from random snapshots of heterogeneous ensembles
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There is mounting evidence that structural variability is common and important to function in biology, and that “structure” is neither static, nor immutable [1-6]. With the exception of NMR, however, current structure determination techniques must often assume the interrogated objects are identical. This includes crystallography, cryo-EM, and the recently burgeoning XFEL-based methods. The study of structural variability and dynamics thus represents an important, but difficult frontier in understanding biological processes. This talk will outline how a new generation of algorithms offers a powerful route to structure and dynamics through random interrogation of members of heterogeneous ensembles.


Keywords: Biomacromolecules, X-ray Free Electron Lasers, Cryo-EM

MS.37.4


Structure determination of biomolecules by XFEL radiation: exploitation of angular correlations of scattered intensities
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The more than billion-fold increase in the brightness over even synchrotron radiation offered by the x-ray free electron laser gives rise to the exciting possibility of biomolecular structure determination from illumination of individual molecules [1], or of small non-crystalline ensembles in solution or in a biomembrane. We will describe progress in developing novel theories for structure determination from this very new form of data.

One approach that has shown much promise is the extraction of structural information from angular correlations of scattered intensities [2,3]. Such an approach offers advantages of efficient compression of the voluminous data produced by high repetition-rate XFEL pulses, of dealing with very noisy data, and of the ability to extract information from disordered biomolecular ensembles closer to those found in nature.

We will describe the latest results of simulations of this approach as well as some tests with experimental data [4]. We also discuss the possibility of exploiting another capability of ultrashort, ultrabright radiation pulses, namely that of determining rapid structural changes of biomolecules during the progress of a light-induced chemical reaction in a more natural environment, such as a solution or a biomembrane, in an experiment in which the molecules are excited by an optical pump beam and interrogated immediately afterwards by an x-ray beam, in analogy with time-resolved crystallography [5].