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Poster Abstracts

Table of Contents & Ballot

Understanding the Role of Charge in Multidrug Recognition and Binding – A First Step in Defeating Multidrug Resistance 2

Conformational hinge in Multidrug transporter activation, N terminus requires Pro72 for effective DNA binding 3

Taking the edge off: The softer side of in-house SAD phasing 4

Structural and Biophysical Studies of Autoinhibited MAP2K MEK6 Dimer 5

The P1 Plasmid Partitioning Complex 6

Structural and Biophysical Characterization of hRecQL4, the DNA Helicase Responsible for Rothmund-Thomson Syndrome 7

Studies on P1 ParA – the Dynamics of Plasmid Partitioning 8

Crystallization of Hfq-RNA complexes 9

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Understanding the Role of Charge in Multidrug Recognition and Binding – A First Step in Defeating Multidrug Resistance

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The emergence of multidrug resistance in various microorganisms poses a significant threat to human health by rendering the current arsenals of antibiotic, antifungal and chemotherapeutics virtually useless. Multidrug resistance (MDR) can be defined as the ability of a cell to withstand normally lethal doses of structurally diverse compounds that are designed to eradicate it. The presence of multidrug resistant bacterial strains such as methicillin-resistant *Staphylococcus aureus* (MRSA), in hospitals is quite alarming. These strains are particularly dangerous for immuno-compromised patients such as cancer patients or a heart transplant recipients undergoing invasive procedures such as multiple surgeries and biopsies. A recent study estimated that 1.2 million patients in the U.S. hospitals alone are infected by MSRA of which approximately 10% infections result in fatalities.

An important contributor to the MDR development in both bacterial and human cells is the multidrug efflux transporters. These transporters are membrane proteins that bind to a wide variety of toxins and drugs and pump them out of the cell thus nullifying their intended action. An understanding of the structural mechanisms of multidrug efflux pumps has been hampered by the difficulty associated with purifying and crystallizing membrane proteins. *In vivo,* the expression level of most MDR transporters is regulated by multidrug binding transcriptional activators/repressors in response to the same toxic compounds that the transporters extrude. These multidrug binding transcriptional regulators are more amenable to structural and biochemical studies as they are cytosolic proteins and can be expressed and purified in large quantities. One such transcriptional repressor that regulates the expression of the *Staphylococcus aureus* multidrug resistance pump QacA is QacR. In spite of various crystal structures that have shown the versatility of this protein to bind different cationic aromatic drugs, the importance of negatively charged residues in the binding pocket is unclear as is their role in modulating drug binding affinity.

The goal of this research is to delineate the role played in drug binding by charged residues present in the binding pocket of a multidrug resistance transcription repressor, QacR, and thereby to elucidate its mechanism of multidrug binding. The implications are multi-faceted as it would give insights into design of drugs, the binding affinities of which can be modulated. Preliminary thermodynamic characterization of drug binding to various QacR mutants is presented in this poster.

Conformational hinge in Multidrug transporter activation, N terminus requires Pro72 for effective DNA binding

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As MtaN is a C-terminal truncation mutant of Mta, it constitutively activates the *mta* promoter and therefore represents a minimal model of transcriptional regulation for the MerR family. As show previously, MtaN contains a flexible hinge (residues 71-75) connecting its DNA binding domain to its dimerization helix, which shows significant conformational adjustment upon DNA-binding (Newberry, 2004). This flexible hinge appears to be characteristic of other MerR family members (Changela, 2003). Importantly, Pro72 appears to impart stereochemical restraints on the hinge region, which allows MtaN to bind its promoter with high affinity. Thus, we hypothesized that mutation of Pro72 to a less constrained residue would significantly diminish MtaN's DNA affinity, even as the hinge is greater that 20 Å from its promoter. Two MtaN mutants, Pro72Ala and Pro72Gly, were created in order to test this idea. DNA-binding experiments and structure determinations of these mutants in their DNA-free and DNA-bound conformations confirmed that Pro72 plays a critical role in maintaining the proper stereochemistry of the hinge region in MtaN for high affinity DNA binding. As a flexible hinge containing a proline residue appears to be a common feature of other MerR family members, these studies further our understanding of signal transduction and energetic costs associated with mutation in the MerR family.

Taking the edge off: The softer side of in-house SAD phasing

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The phase problem in macromolecular crystallography has been mitigated dramatically in recent years by advances in methodology and instrumentation. SAD phasing has now become the primary *de novo* phasing method. A search of the PDB of structures released in 2006 reveals the number of structures solved by SAD phasing exceeds those solved by MAD for the first time. A number of these examples of successful S-SAD and Se-SAD phasing used Cr radiation (? = 2.29 Å), which can double the anomalous signal of sulfur and selenium compared to Cu radiation.

This report reviews recent results from phasing with the enhanced anomalous signal provided by Cr radiation to demonstrate this longer wavelength can be used to solve *de novo* structures. Selenium, as the heavy atom, with Cr radiation can provide sufficient anomalous scattering for routine phasing. Cr radiation opens a new path to extracting the weak anomalous signal from sulfur to phase native protein data. With the addition of Cr radiation to the crystallographer's toolkit, in-house X-ray sources can routinely provide at least two wavelength options. The combination of diffraction data collected using both Cu (? = 1.54 Å) and Cr radiation can improve the electron density tremendously. Anomalous scattering from sulfur can also assist in molecular replacement solutions. Finally, the data collected with Cr radiation can be used to refine a structure. Ultimately, this makes it possible to solve a protein structure with a single data set. This in-house phasing approach we describe has been given the label "**know before you go**" by John Rose and B.C. Wang at the University of Georgia. This method improves the efficiency of the solution of macromolecular crystal structures and usage of the synchrotron beam time.

Structural and Biophysical Studies of Autoinhibited MAP2K MEK6 Dimer

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MAP2Ks are dual-specificity protein kinases functioning at the center of three-tiered MAP kinase modules. The structure of kinase domain of MEK6 MAP2K possessing phosphorylation site mimetic aspartic acid mutations (MEK6/? N/DD) has been solved at 2.3 Å by X-ray crystallography. The structure reveals an elongated ellipsoid of an autoinhibited dimer. The conformation adopted is inactive, despite the phosphate-mimetic mutations. Gel filtration, small angle X-ray scattering (SAXS) analysis, and mutagenesis confirm the crystallographically observed ellipsoidal dimer is a feature of full length unphosphorylated and phosphorylated wild-type MEK6 in solution. The interface includes the phosphate binding ribbon of each subunit, part of the activation loop, and a rare "Arginine Stack" between symmetry related arginine residues in the N-terminal lobe. The C-terminus of MEK6 also makes contacts in the interface. The autoinhibited structure of a MAP2K likely confers specificity on active MAP2Ks. The dimer may function in unphosphorylated MEK6 to prevent its phosphorylation.

The P1 Plasmid Partitioning Complex

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Faithful segregation of DNA is an essential process in all organisms. In prokaryotes segregation of low copy number plasmids is performed by partitioning systems, generally termed *par*. Although much is known of the mechanisms involved in segregation of DNA in eukaryotes, the mechanism by which this process occurs in prokaryotes has yet to be fully determined. The partitioning system of the archetypal P1 plasmid is one of the best characterised. The P1 *par* operon encodes a Walker-type ATPase, ParA, and a DNA binding protein, ParB, which assembles at the plasmid partitioning site, *parS*. The P1 partitioning complex is formed by the binding of ParB and the host encoded integration host factor (IHF) at specific sites within *parS*. The crystal structures of both ParB and IHF bound to DNA have previously been published. The aim of this project is to elucidate the structure of the partitioning complex containing ParB and IHF bound to the same DNA fragment. This will be attempted using traditional crystallographic methods and small angle X-ray scattering (SAXS). SAXS provides the radius of gyration of a molecule or molecular complex and with enough data permits determination of a low resolution (10-20 Å) model of a macromolecular structure. These data, in conjunction with high resolution structures of ParB and IHF bound to their respective DNA sites, will be used to obtain structures of several ParB-IHF-DNA complexes. SAXS is also a complementary method to crystallography as data are collected in solution. SAXS data have given an indication of the radii of gyration of complexes using different lengths of DNA. Different possible models of the partitioning complex are being tested for level of fit to SAXS data.

Structural and Biophysical Characterization of hRecQL4, the DNA Helicase Responsible for Rothmund-Thomson Syndrome.

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Mutations to the human DNA helicase, hRecQL4, give rise to genomic instability that in turn is responsible for a group of autosomal recessive conditions including Rothmund-Thomson Syndrome, RAPADILINO and the majority of cases of Baller-Gerold Syndrome. The symptoms and severity of each syndrome are correlated to mutations that broadly map to the helicase and C-terminal regions of hRecQL4 and their effects are poorly understood. hRecQL4 is classified as a RecQ family helicase that also includes the Werner's (WRN) and Bloom's (BLM) Syndrome helicases which function during DNA repair and replication restart. Recent work from our lab and that of our collaborators (A. Venkitaraman, Cambridge U.) suggest that although there are some similarities to WRN and BLM, hRecQL4 plays an essential role in the initiation of DNA replication and has a quaternary structure unlike WRN and BLM. Since over 50% of the hRecQL4 primary sequence shows no significant homology to any known protein, we used a structural genomics approach to design a number of expression constructs. Our recent studies focus on the structural determination of hRecQL4 constructs containing the conserved helicase domain and constructs containing the Sld2-like domain. The constructs that contain the helicase domain elute as a single peak at a molecular weight range of 443-2000 kD consistent with the size expected for an oligomeric species and are able to hydrolyze ATP. Currently we are screening crystallization conditions for the human RecQL4 constructs containing the Sld2-like domain as well as the corresponding constructs from the *Mus musculus* and *Xenopus laevis* homologs. These data together with the unique clinical phenotypes associated with mutation of hRecQL4 suggest a distinct biochemical function for hRecQL4.

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Studies on P1 ParA – the Dynamics of Plasmid Partitioning

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An essential feature of living cells is the ability to pass-on genetic information to succeeding generations. *E.coli* and other bacteria have systems insuring the competent segregation of plasmids and chromosomes based upon specific protein-DNA and protein-protein interactions. The low copy number P1 plasmid is stably maintained in *E.coli* through the interactions of two plasmid-encoded proteins, ParA and ParB. ParA, an ATPase containing a deviant Walker A ATPase domain, is essential for plasmid translocation in a yet to be understood manner. Recent studies on the P1 ParB protein have elucidated its role as a DNA binding protein responsible for pairing newly replicated plasmids [1]. The next key step to understanding partitioning in the P1 system is obtaining structural information on ParA that will provide clues to how its ATPase function and interaction with ParB leads to the distribution of genetic material to each daughter cell.

Efforts to elucidate the mechanism behind plasmid partitioning lead to the discovery that ParA forms filamentous structures in the presence of ATP. In a simple reconstituted system, ParA alone, we observed filaments that were many microns in length projecting in several directions. With the addition of the other essential components of the partition complex, ParB and ParS (the plasmid centromere), the filaments were concentrated between nodes comprised of these new components. These observations provide visual evidence for ParA's role as the physical driver of partitioning - acting to push apart the newly paired plasmids. With future experiments, we hope to concentrate on the dynamic aspects of filament formation in an effort to address mechanistic questions of how ParA might fulfill its role in partitioning.

[1] Schumacher MA, *et al*. (2005) *Nature*. 438: 516-519

Crystallization of Hfq-RNA complexes

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Hfq is a post-transcriptional regulator that governs many important cellular functions and the deletion of it causes pleiotropic effects including decreased growth rates and increased sensitivity towards a variety of stress conditions. Hfq forms hexamers and preferentially binds to poly(A) and single-stranded AU rich sequences. It was established as an RNA chaperone. By concomitant binding of several small non-coding RNAs (sRNA) and target mRNAs Hfq enables the formation of stabile sRNA-mRNA hybrid duplexes thereby adjusting the translation rate. Further, Hfq was shown to modulate the decay of some mRNAs by binding their poly(A) tails and protecting them from degradation by enzymes forming the RNA degradosome. Despite several genetic and biochemical investigations our mechanistic understanding of Hfq function is limited due to the lack of relevant high resolution structures. Beside the three Hfq apo structures from *S. aureus*, E. coli and *P. aeroginosa* only the structure of *S. aureus* Hfq bound to the hepta-oligoribonucleotide AU5G was solved. It reveals a ring-shaped structure with a central pore and high similarity to Sm proteins. In that structure the RNA is bound on the proximal site and expands and fills the central basic pore. Biochemical data have postulated an additional distal RNA binding site for poly(A) tails but the model for this protein-RNA interaction is still speculative and affords structural investigations. Furthermore, recent data in our lab unveiled discrepancies between the RNA binding affinities of Hfq from *E. coli* and *S. aureus* and an intriguing reverse salt effect of *S. aureus* Hfq binding to A6 and U6 RNAs indicating different binding modes (T. Link and R. G. Brennan, unpublished results). To understand the underlying mechanism of RNA binding by Hfq, we crystallize *S. aureus* Hfq in complex with A6 RNA and A16 RNA. Crysta! llization and preliminary diffraction data are shown.