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MS04 P17

The Center for Structural Molecular Biology (CSMB) at Oak Ridge National Laboratory (ORNL) G.W. Lynn W.T. Heller, A. N. Raghavan, V. S. Urban, K.L. Weiss, Y. Mo and D.A.A. Myles, *Chemical Sciences Div. ORNL, Oak Ridge, TN 37831*. E-mail: lynngw@ornl.gov

Keywords: small-angle neutron scattering, biology, biomembranes,

The CSMB at ORNL is developing facilities and techniques for the characterization and analysis of biological systems at the High Flux Isotope Reactor (HFIR) and the Spallation Neutron Source (SNS). The cornerstone of the effort is a small-angle neutron scattering instrument (Bio-SANS) at HFIR that will be dedicated to the analysis of the structure, function and dynamics of complex biological systems. In support of this program, we are developing advanced computational tools for neutron analysis and modeling, alongside a supporting biophysical characterization and X-ray scattering infrastructure. Specifically, we established a Bio-Deuteration Laboratory for *in vivo* production of H/D labeled macromolecules that will permit selected parts of macromolecular structures to be highlighted and analyzed *in situ* using neutron scattering. The CSMB is also expanding our efforts to include the study of biomembranes by neutron reflectometry. These new facilities will make ORNL a world-leading scientific center and user facility for neutron-based studies of bio-molecular structure and function.

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MS04 P18

Structure of IgNAR single domain antibody and *Plasmodium falciparum* AMA1 complex Victor Streltsov[a], Kylie Henderson[a,b,c], Andrew Coley[b,c], Olan Dolezal[a], Adrian Batchelor[d], Vincent Murphy[c], Robin Anders[3], Michael Foley[b,c], Stewart Nuttall[a]. [a]*CSIRO Molecular and Health Technologies, Melbourne, Australia.* [b]*Cooperative Research Centre for Diagnostics, Brisbane, Australia.* [c]*School of Biochemistry, La Trobe University, Melbourne, Australia.* [d]*University of Maryland School of Pharmacy, Baltimore, Maryland, USA.* E-mail: victor.streltsov@csiro.au

Keywords: three-dimensional protein structure; antibody antigen complexes, biological macromolecular crystallography

Apical Membrane Antigen-1 (AMA1) is essential for red blood cell invasion by *Plasmodium* parasites and is a leading malarial vaccine candidate. However in humans several infection cycles are required to establish AMA1-specific protective immunity due to extensive polymorphisms within the protein's surface-exposed loops. Using an AMA1-specific IgNAR (Immunoglobulin New Antigen Receptors) single variable domain antibody as starting material, we performed targeted mutagenesis and iterative selection against AMA1 proteins from *Plasmodium falciparum* strains 3D7, W2mef, and HB3. We present the co-crystal structures of two resulting antibody-AMA1 complexes, which reveal the extended IgNAR CDR3 (Complimetarity Determining Region) loops penetrating deep into a hydrophobic cleft on the antigen surface, and contacting residues conserved across parasite species. Comparison of a series of CDR3-based affinity-enhancing mutations allowed dissection of the relative contributions to binding kinetics of various CDR3 – AMA1 contacts, and correlation of these affinities to inhibition of parasite red blood cell invasion. Taken together, these findings and structures provide insights into the mechanisms of single-domain antibody binding, and will enable future design of reagents which target otherwise cryptic epitopes in apicomplexan parasites.

MS04 P19

X-ray Absorption Spectroscopy Study of Copper Binding to β–Amyloid Peptide Victor Streltsov[a], Kevin Barnham[b], Jose Varghese[a], [a]*CSIRO Molecular and Health Technologies, Melbourne, Australia.* [b]*University of Melbourne, Melbourne, Australia.*
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Keywords: beta-amyloids, copper proteins, EXAFS

While the causes of Alzheimer's disease (AD) are still uncertain, the deposition of misfolded protein, described as amyloid plaque, is considered as defining pathological feature of AD. The major constituent of AD plaques is the β-amyloid peptide (Aβ) that is cleaved from the membrane-bound amyloid precursor protein. *In vitro*, Aβ binds metal ions including Cu^{2+} giving rise to extensive redox chemical reactions. Since elevated levels of Cu are found in amyloid deposits in AD affected brains, the oxidative stress causing cellular damage may be related to the production of reactive oxygen species by metallated forms of Aβ [1,2]. A number of studies indicated that the coordination sphere around the Cu ions is nitrogen rich and different types of coordination has been proposed for ligands to Cu ions in Aβ Cu complexes. The intrinsic propensity of Aβ to self-association creates experimental obstacles and may lead to different Cu binding geometries observed. Preparation of protein samples with structural homogeneity is critical [3]. A series of X-ray Absorption Spectroscopy (XAFS) studies on β–Amyloid peptide Cu complexes under a range of conditions are presented.

[1] Bush, A.I. *TINS*, 2003, 26, 207. [2] Curtain C.C. et al., *JBC*, 2001, 276, 20466. [3] Teplow, D. B. *Methods in Enzymology*, 2006, 413, 20.

MS06 P12

Facilitating Low Volume Protein Crystallography Set-ups Using the mosquito® Liquid Handler. Joby Jenkins, Rob Lewis, Jas Sanghera, Chloe Milburn
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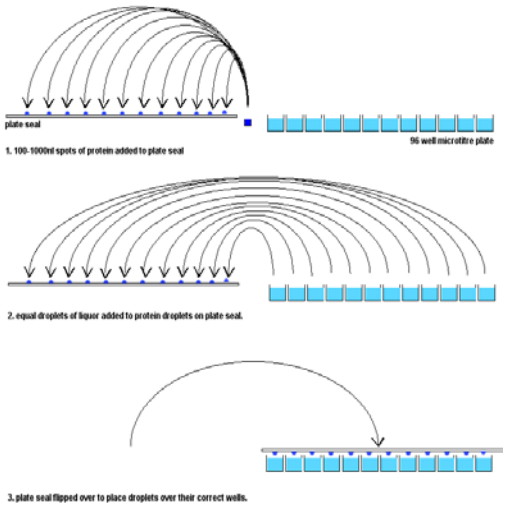
A prerequisite for efficient high throughput protein crystallisation screening is the accurate pipetting and positioning of the low volume drops used in hanging and sitting drop setups. Screening the many different conditions under which a protein crystal may form lends itself to automation, since it requires hundreds of similar experiments to be set up to find the few 'hits'. Automated solutions exist for low volume pipetting, however, the variable viscosities of protein and reservoir/screen solutions present significant challenges for many liquid handling systems. Another challenge is that of drop positioning. The mosquito® (TTP LabTech) offers fast positive displacement pipetting for accurate and reproducible aspiration and dispensing throughout the 50 nL - 1.2 µL range, producing CVs of <8% at 50 nL irrespective of viscosity. This, plus its columnar arrangement of pipettes, allows it to automate hanging drop as well as sitting drop set-ups. Mosquito's micropipettes are also disposable, thus guaranteeing zero cross-contamination where required.

Figure: Automated hanging drop setup



MS06 P13

Development of a new microplate for micro-scale vapour diffusion. Marek Brzowzowski1, Justyna Korczynska1, Ting-Chou Hu1, David K Smith1,Joby Jenkins2, Rob Lewis2

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The automation of the crystallisation process has contributed significantly to the rapid progress of crystallography-based structural biology. For example, 96-well plates have been seamlessly incorporated into automated protein crystallography set-ups enabling much higher process throughputs. This development has delivered a plethora of crystallization plates suitable for both automated and manual set-ups. However, practically all these plates (except microfluidic channel chips) are based on a very similar design and well volume to drop ratios (50-100uL to 25-150nL).
TTP LabTech and the York Structural Biology Laboratory have pooled their expertise in engineering and protein crystallography to develop and test a new type of crystallization plate (μplate) that still employs classical vapour diffusion technique but minimizes the precipitant well volume down to 1.2-10µL. This enables:

- a very significant saving on the total bulk of screens

the use of rare and chemically expensive solutions for automated screening procedures.

MS09 P04

Structure and activity of Kunjin virus NS3 helicase domain Eloise Mastrangelo[a], Mario Milani[a], Michela Bollati[a], Graziella Sorrentino[a], Bruno Canard[b], Dmitri I. Svergun[c] and Martino Bolognesi[a] [a]*Department of Biomolecular Sciences and Biotechnology, University of Milano, Italy.* [b]*Laboratoire Architecture et Fonction des Macromolécules Biologiques, AFMB-CNRS-ESIL, Marseille, France.* [c]*E.M.B.L. Hamburg, Germany*
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Keywords: helicase structure; Kunjin virus; flavivirus NS3 protein

Flaviviral NS3 is a multifunctional protein displaying N-terminal protease activity in addition to C-terminal helicase, nucleoside 5'-triphosphatase (NTPase), and 5'-terminal RNA triphosphatase (RTPase) activities. NS3 is held to support the separation of RNA daughter and template strands during viral replication. We solved the three-dimensional structure of the NS3 helicase domain (residues NS3:186-619) from Kunjin virus, an Australian variant of the West Nile virus. As for homologous helicases, NS3:186-619 is composed of three domains, two of which are structurally related and held to host the NTPase and RTPase active sites. The third domain is involved in RNA binding/recognition. Normal mode analysis of the NS3:186-619 helicase construct indicates the presence of a scissors-like oscillation involving domains II and III, resulting in opening/closure of the ssRNA binding cleft entrance. Such intramolecular scissors-movements may be part of the inchworm mechanism by providing a strain component for dsRNA unwinding [1]. NS3:186-619 displays both ATPase and RTPase activity and can unwind a dsRNA substrate. Analysis of different constructs shows that full length NS3 displays increased helicase activity, suggesting that the protease domain plays an assisting role in the RNA unwinding process. The structural interaction between the helicase and protease domain has been assessed using small angle X-ray scattering on full length NS3, disclosing that the protease and helicase domains build a rather elongated molecular assembly differing from that observed in the NS3 protein from HCV.

[1] Mastrangelo, E. et al. (2007). Crystal structure and activity of Kunjin Virus NS3 Helicase; Protease and Helicase Domain assembly in the Full Length NS3 Protein. *JMB.* [Epub ahead of print]

MS13 P29

Energetic and structural studies in

thiazolidines-2-thiones series and its comparison with a conformational study. A. LAKNIFLI, A. HAMINE, A. ELHAMMADI. Department of Chemistry, University of Ibn Zohr, Faculty of Sciences, PO 8106, Agadir, *Morocco.* E-mail: abdelaknifli@yahoo.fr