Shapiro L, Martinis SA, Benkovic SJ, Cusack S, Alley MR. Science. 2007 Jun 22;316(5832):1759-61

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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