novel, biologically interesting and crystallization-feasible targets that were then designed into 96 different constructs. Processing of the 96 constructs was performed in parallel using simple automated applications of ligation-independent cloning, small-scale bacterial expression and purification, and solubility assessment. After processing the 96 constructs of 12 targets, I found that 16 constructs of three targets (25%) yielded soluble protein. From the three soluble targets, I have spent most time on two of these protein.

The first protein is a CARD domain containing protein that interacts with Bcl10. The primary function of Bcl10 is to interact with CARD proteins through CARD-CARD interactions to regulate its activity [2]. The crystal structure of this CARD containing protein solved at 1.5 Å resolution revealed six anti-parallel α-helices, showing that this protein is indeed similar to other CARD proteins with known structures. Approaches to determine the interaction between these two CARD domain containing proteins are currently being applied.

The second protein I worked on is a DUF59 domain containing protein with no function characterized yet. However, it has been reported that a family member is part of the MMXD protein complex involved in chromosome segregation [3]. I solved two crystal structures of this DUF59 domain protein to 1.8 Å resolution revealing, unusually, two different types of domain swapped-dimer. Functional characterization of this DUF59 domain containing protein, and of its domain swapping, is currently being investigated.

[1] M. Puri, N. Cowieson, J.K. Forwood, P. Listwan, S.H. Hu, G. Guncar, T. Huber, S. Kellie, D.A. Hume, B. Kobe, J.L. Martin, *Biomolecular Engineering* **2006**, *23*, 281-289. [2] J. Ruland, G.S. Duncan, A. Elia, I.D.B, Barrantes, L. Nguyen, S. Plyte, D.G. Millar, D. Bouchard, A. Wakeham, P.S. Ohashi, T. W. Mak, *Cell* **2001**, *104*, 33-42. [3] S. Ito, L. J. Tan, D. Andoh, T. Narita, M. Seki, Y. Hirano, K. Narita, I. Kuraoka, Y. Hiraoka, K. Tanaka, *Molecular Cell* **2010**, *39*, 632-640.

Keywords: structural_genomics, crystallography, macrophage

MS50.P12

Acta Cryst. (2011) A**67,** C547

Deciphering the mechanisms responsible for promiscuity in primary humoral response

Tarique Khan,[a] Dinakar M Salunke,[a],[b] [a]*Structural Biology Unit, National Institute of Immunology, Aruna Asaf Ali Road, New Delhi 110067, (India).* [b]*Regional Centre for Biotechnology, Gurgaon 122016, (India).* E-mail: tarique@nii.ac.in

The antigenic repertoire is infinite. In order to generate an effective immune response, every antigen has to be specifically recognized in the primary encounter so that appropriate immune response could be mounted. In terms of the physico-chemical principles of antigen-antibody recognition, charge and shape complementarity is the key feature of antigenic discrimination and therefore, the number of antibodies required to neutralize the infinite population of antigens, ought to be unlimited. The fact that the germline antibody repertoire is finite (being limited by the number of temporal and spatial B cells and fixed recombinatorial potential of VDJ gene segments) implies that the germline antibodies could be broadly polyspecific in order to fulfill the physiological requirements of the primary immune response. While emerging data appear to invoke a paradigm shift on how Ag recognition is actually achieved in a primary humoral response, the structural mechanisms for promiscuous binding capabilities of germline antibodies have not been yet clearly illustrated.

Towards understanding the mechanistic basis for multispecificity in primary humoral response, structure and binding modes of a germline mAb BBE6.12H3 with multiple independent antigens were examined at atomic resolution. Our study demonstrates conformational flexibility of BBE6.12H3 paratope both in antigen-bound and antigen-free states. CDRH3 could undergo conformational rearrangements to adapt to independent and structurally different peptides. Six crystal structures of BBE6.12H3 illustrate diversity of antigen recognition repertoire and provide structural evidence for correlation of paratope flexibility with the multispecificity of germ line antibody. Moreover, comparative analysis of interacting residues in these complexes suggested that antigen combining site may be predesigned to be polyspecific. It is proposed that of primary antibody repertoire involves large, yet, finite germ line antibody clones, each capable of adopting discrete conformations which, in turn, show diverse binding modes.

Keywords: humoral immunity, immune recognition, paratope flexibility

MS50.P13

Acta Cryst. (2011) A**67,** C547

Crystal structure of CEL-IV, isolated from a sea cucumber, cucumaria echinata

Hideaki Unno,[a] Tomomitsu Hatakeyama,[a], Takuro Kamiya,[a], Masami Kusunoki,[b] Sachiko Nakamura-Tsuruta,[c] Jun Hirabayashi, [c] Shuichiro Goda,[a] [a]*Department of Applied Chemistry, Faculty of Engineering, Nagasaki University, Nagasaki (Japan).* [b]*Graduate School of Medicine and Engineering, University of Yamanashi, Kofu (Japan).* [c]*Research Center for Medical Glycosciences, National Institute of Advanced Industrial Science and Technology, Tsukuba (Japan).* E-mail: unno@nagasaki-u.ac.jp

CEL-IV is a C-type lectin isolated from a sea cucumber, Cucumaria echinata. This lectin is composed of four identical C-type carbohydrate-recognition domains (CRDs). X-ray crystallographic analysis of CEL-IV revealed that its tetrameric structure was stabilized by multiple interchain disulfide bonds among the subunits [1]. Although CEL-IV has the EPN motif in its carbohydrate-binding sites, which is known to be characteristic of mannose binding C-type CRDs, it showed preferential binding of galactose and N-acetylgalactosamine. Structural analyses of CEL-IV-melibiose and CEL-IV-raffinose complexes revealed that their galactose residues were recognized in an inverted orientation compared with mannose binding C-type CRDs containing the EPN motif, by the aid of a stacking interaction with the side chain of Trp-79. Changes in the environment of Trp-79 induced by binding to galactose were detected by changes in the intrinsic fluorescence and UV absorption spectra of WT CEL-IV and its site-directed mutants. The binding specificity of CEL-IV toward complex oligosaccharides was analyzed by frontal affinity chromatography using various pyridylamino sugars, and the results indicate preferential binding to oligosaccharides containing Gal-beta-1–3/4(Fuc-alpha-1–3/4)GlcNAc structures. These findings suggest that the specificity for oligosaccharides may be largely affected by interactions with amino acid residues in the binding site other than those determining the monosaccharide specificity.

[1] T. Hatakeyama, T. Kamiya, M. Kusunoki, S. Nakamura-Tsuruta, J. Hirabayashi, S. Goda, H. Unno, *J. Biol. Chem.,* **2011,** *286(12)*, 10305-10315.

Keywords: CEL-IV, lectin

MS50.P14

Acta Cryst. (2011) A**67,** C547-C548

Structural analyses of mouse MD-1 protein complexed with endogenous phospholipid

Umeharu Ohto, Hitomi Harada, and Yoshinori Satow, *Graduate*