EDITORIAL

Broadening horizons of antibody engineering

This 2011 Antibody Special Issue continues the annual tradition that PEDS adopted after becoming the Official Journal of The Antibody Society. Many research papers in this issue involve important facets of display library research. This emphasis coincides with the 20th anniversary of the first definitive publications on construction and selection of human phage display antibody libraries by Marks et al. (1991) (naïve library) and Burton et al. (1991) (disease-specific library), with related papers cited from each research group, and the 21st anniversary of the first phage antibody display library (McCafferty et al., 1990), comprising single-chain Fv (scFv) binding sites. This issue demonstrates how the recombinant antibody and related cellular immunology fields remain creative and vigorous after a generation of singular advances. The triumphs of this era of research are mirrored by the remarkable success of therapeutic antibodies since the late 1990s. Immunotherapy has saved or extended countless lives and provides for important new clinical opportunities. This global research effort continues to broaden the horizons of antibody engineering and its medical impact.

This Special Issue allows an integrated focus on the antibody field within a single collection of papers, although throughout the year PEDS publishes important investigations on the engineering, design and selection of antibody and related binder proteins. For instance, the first two papers of this issue (a Letter to the Editor and the Response) refer to a comprehensive paper published in the March 2011 issue. This thoughtful discussion emphasizes how PEDS provides a mechanism for the discussion of its papers. The Antibody Society likewise seeks to develop forums for discussion to enhance the vitality of our field, and therefore promotes direct scientific interactions at our Annual Meeting, starting with 2010. Our website (http://www.antibodysociety.org) describes the meeting, links to online registration, notes 2011 student award opportunities and cites members’ large discounts for Annual Meeting registration and Society journal subscriptions. It is also a rich source of new information on commercialized mAbs, those en route to the clinic, and ongoing Society initiatives. Furthermore, it provides a link to the published summary of our Annual Meeting, starting with 2010.

The Letter to the Editor and its Response are followed by two papers that assess protein and antibody dynamics. (1) Ozbabacan et al. review transient protein—protein interactions, the dynamics of protein association and analysis of weak binding. This reinforces an appreciation for the existence of meaningful binding specificity despite weak association (i.e. with a Kd of micromolar or less), as well as for regulated systems such as microtubules that are purposefully switched between supramolecular assemblies and disassociated subunits during different stages of cell division. These considerations increasingly apply to our field, given a growing interest to engineer antibody-mediated targeting of cellular or nanoparticle delivery vehicles and to construct diverse multivalent binding molecules. The immune system takes advantage of such transient binding phenomena in its initial recognition of antigens by IgM, or low-affinity binding of MHC class II-peptide complexes (pMHCII) by T-cell receptors (TCRs). (2) Wang and Duan cover a related aspect of protein interactions, using molecular dynamics simulations to identify the sites limiting structural stability in an anti-VEGF scFv. Their general conclusions are broadly parallel to those of thermodynamic studies in the literature. This analysis arrives at specific proposals for alteration of VH and VL domain residues and interdomain contacts to enhance stability of the anti-VEGF scFv.

Three papers involve membrane proteins in the studies of receptor binding, its modulation by engineering of the binding protein, and antibody phage display selection of integral membrane proteins. (1) The paper by Holla and Skerra is a comprehensive study of very weak, but critical, interactions of particular mono- and disaccharides with the monomeric binding sites of DC-SIGN and Langerin. These are dendritic cell surface proteins that interact with glycan structures of HIV-1 to facilitate cell-specific HIV entry. A detailed binding analysis with carbohydrate microarrays coupled with isothermal titration calorimetry allowed them to measure Ca2+-dependent, specific binding to internal mannose residues (DC-SIGN) or a more complex panel of terminal sugars (Langerin) with Kd values in the mM range. (2) The paper by Stewart et al. addresses the improvement of antibody-dependent cellular cytotoxicity with therapeutic antibodies by engineering a variant of the IgG1 human Fc region. Ribosome display was utilized to select for improved Fc binding to the FcyIIIA receptor of NK cells. Using highly sophisticated selection methods, they ultimately narrowed their mutants to an optimal version. This 125_B01 variant had mutations that favored the absence of fucose or bisecting N-acetylgalactosamine within the expressed CH2 domain, and also incorporated a Phe243Leu replacement that is a carbohydrate contact residue. (3) Hötz et al. dealt with the efficient production of antibodies to integral membrane proteins. Using an Fab-phage display library coupled with membrane protein target production in bacculovirus, they selected binders to claudin-1, a co-receptor for cellular entry of hepatitis C virus (HCV). Affinity maturation resulted in an optimized Fab that inhibited HCV infection. Their approach could prove useful to increase access to membrane protein targets for antibody library selection.

Four investigations are devoted to intriguing aspects of phage display library research. (1) Brockmann et al. describe a rapid approach for affinity maturation with VH, chain shuffling after selection with a single-framework (VH3-23) scFv-display library. This carefully documented approach is proposed to access greater library diversity than is possible with conventional approaches. (2) The noteworthy investigation of Wen et al. compares yeast and bacculovirus cell display of functional human MHC class II proteins bound to myelin basic protein, pMHCII [a single-chain construct with DR2: DR2α-L-P-L-DR2β (Fig.1a)]. Each system has its advantages, but only the insect cell display system allowed the directed evolution of MHCII to obtain DR2 variants with improved binding affinity for TCRs. This investigation...
creates new opportunities for therapeutic and diagnostic uses of functional major histocompatibility class II proteins. (3) Patel et al. describe a combination of phage and yeast display, which they term parallel selection with a cross-species display platform. It offers advantages in dramatically increasing the total number of unique clones obtained, compared with either system alone. They also devised a novel C-terminal adapter that permits their Fab library-derived binders to be expressed in either display system and facilitates their ‘cross-phage/yeast display vector’ to be readily shuttled between platforms. (4) Noppe et al. discovered apparent heterogeneity upon expansion of single-phage clones selected from peptide-phage libraries, in that the numbers of peptides genetically fused to the pIII protein of filamentous phage appeared to be quite variable. This was determined using supermacroporous chromatographic gels with immobilized target molecules (human lactoferrin, HuLF), through which 6-mer-peptide-phage particles could readily flow and interact with HuLF. The novel immunoadsorbent column separated the phage particles into weak, medium and strong binders.

Three papers involve single V domain antibodies (nanobodies) and/or related advances relevant to crystal structure determination of nanobody–antigen complexes. (1) Rahma et al. engineered a humanized version of a nanobody (Nb) with improved neutralizing capacity against AahII scorpion toxin. Starting with NbAahII10 they also removed an unpaired cysteine residue from CDR1 of the original camel Nb, choosing Cys38Ser as their optimal substitution. This may provide a potent antidote to this particularly deadly scorpion toxin and offers a model for development of similar Nb agents for neutralizing toxins. (2) Absharom et al. describe a new combination of crystallization methods to obtain high-resolution co-crystals of non-pathogenic prion protein (PrP\(^\text{C}\)) complexed to a PrP\(^\text{C}\)-specific Nb. When no usable crystals were formed and subjected to limited proteolytic digestion in solution, the method failed to produce crystals. Thus, a new step was added to the process, where Nb-PrP\(^\text{C}\) complexes were formed and subjected to limited proteolytic digestion in solution. Segments of the PrP\(^\text{C}\) proteins resistant to digestion remained bound to the Nb. These truncated complexes were crystallized, but gave only poor resolution. Nonetheless, these crystals were mechanically pulverized to create nucleation sites for a new round of MMS. The original mixtures of intact PrP\(^\text{C}\) and Nb proteins were combined with these crystal fragments to nucleate improved crystals. This resulted in very high-resolution crystals of the intact proteins that allowed synchrotron data collection suitable for a 1.5 Ångstrom structure determination. This approach may be extended to the crystallization of other antigens complexed with specific Nb or scFv species that are protease resistant. (3) The amyloid beta (A\(\beta\)) peptide component of the plaque in Alzheimer’s disease is of great interest for crystallization and structural analysis. However, A\(\beta\) aggregation creates a barrier to such studies unless the heterodimer can be stabilized, for example, in an Nb or scFv complex. In the paper by Broersen et al. this goal may have been given impetus with their development of a protocol for preparation of aggregate-free A\(\beta\) peptide. This yields high-quality preparations that appear suitable for biological and biophysical studies.

The final two papers in this issue investigate novel scaffolds that further augment the active area of antibody-like single-scaffold binders. (1) Stadler et al. describe an important variant of a new class of single-scaffold proteins termed Scannins. These ~100 residue scaffolds have the attractive property that they can allow the genetic incorporation of distinct aptamer peptides on each of three different faces of the protein. Their extensive structure-function investigation provides a solid basis for the use of Scannins in diverse applications that include combinatorial display libraries. (2) Anderson et al. address the phenomenon of structural plasticity in proteins with their designed ‘chameleone sequence’ (CS) that adopts either an \(\alpha\)-helical or \(\beta\)-sheet backbone conformation depending upon its N-terminal fusion partner. As a fusion to the Cro C-terminal subdomain, P22 Cro-CS has \(\alpha\)-helix for CS, while \(\lambda\) Cro-CS has \(\beta\)-sheet for the CS sequence. These fusion constructs and several CS sequences were extensively characterized by biophysical methods, including \(^1\text{H}-^1\text{H}\) NMR correlation spectroscopy, which indicates that the 24-residue CS and its newer 19- and 22-residue truncated forms all exhibit the ability to undergo smooth structural transitions. This model system could improve understanding of protein evolution and protein misfolding, relevant to prion-based and other major neurodegenerative diseases. Protein binder applications are peripheral at this juncture, but the availability of a switch sequence in a protein could be important in future protein or antibody engineering.

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The Antibody Society thanks its members and sponsors for supporting our 501(c)(6) non-profit Society. We encourage maintaining active membership at our website for graduate students (registrations is free), postdoctoral fellows, and full members, who are all vital to the success of our Society. Corporate sponsors provide essential financial support and a bridge to the private sector, for which they are gratefully acknowledged on our website (http://www.antibodiesociety.org). This remains our Village Green, where we communicate valuable information that emerges each month and identify key resources for our international community.

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References