

**P012** Application of chelating recombinant antibody phage libraries  
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There are a host of elaborate approaches for improving recombinant antibody affinity. If the atomic structure of the antibody-antigen complex is known, mutations can be introduced to optimise protein-protein interactions. In the absence of such detailed structural knowledge, random mutations followed by a selective technique can yield higher affinity binders. An alternative approach was described by Neri et al. where two scFvs which bound to non-overlapping epitopes of the same antigen molecule were fused by a peptide linker in such a way to allow simultaneous binding (i.e. antigen 'chelation') resulting in an affinity enhancement of 10-100 fold (Chelating Recombinant Antibody-CRAB). This was achieved knowing the crystal structures of each scFv bound to lysozyme. We describe here a robust and widely applicable approach to link *any* pair of scFvs using a library of peptide linkers, followed by phage display experiments to select for the pair with the correct linker length enabling antigen chelation, without the need for structural knowledge. Using this CRAB library approach, we show that each individual scFv of the CRAB species can be displayed on phage and bind to its antigen molecule. Selection experiments show that careful control of antigen concentration is important to produce conditions where competition is high leading to discrimination of chelating binders. These results, exemplified with the antigens lysozyme and interleukin-6 open up CRAB technology to many applications which will be discussed.