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Probing Binding Mechanism of Interleukin-6 and olokizumab: *In Silico* Design of Potential lead Antibodies for Autoimmune and Inflammatory Diseases

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Commentary

Computer aided drug design has rationalized drug discovery process-from identification of putative drug targets to structure based lead discovery and optimizations. It includes screening of differentially expressed genes in disease conditions and imputing potential drug targets through pathway analysis, gene ontology enrichment analysis and gene network analysis. Molecular modelling techniques (protein structure modelling and molecular dynamics simulations) subsequently aid in determining tertiary structures of these potential drug targets closer to their native conformations. The functionally important binding pockets or protein-protein interacting sites in the tertiary structure of drug targets are the intriguing starting point of structure based drug design. Although, both small molecule inhibitors and antibody based therapeutic discovery techniques have been widely used as in drug discovery programs; antibody based therapies (biologics) are of significant interest due to their specificity towards a particular drug targets. Nevertheless, biologics have showed better efficacy in successful remission of autoimmune and inflammatory disease. Furthermore, site directed mutagenesis inducted on known antibodies have been shown to improve their binding affinity by ~10 – 454 folds towards their desired target proteins *in vitro* [1–4]. These experimental insights, in fact encouraged us to implement molecular modelling approaches to explore if there exist any scope for improving existing therapeutic axis *in silico*.

In this regard, we have selected interlukin-6 - olokizumab antigen-antibody (Ag-Ab) complex tertiary structure to implement a systematic protocol of computer aided antibody design (Figure 1) [5]. Interlukin-6 (IL-6) is one such pleiotropic cytokine that regulates several branches of immune system. Although IL-6 has numerous beneficial contributions like differentiation of T- helper cells, effect on hematopoietic cells, its continuous production sidetracked to various autoimmune and chronic inflammatory diseases. Therefore, IL-6 have been implicated as a potential drug targets for autoimmune and inflammatory diseases. Olokizumab is a neutralizing antibody of IL-6. It down regulates IL-6 expression by interacting at site III to restrict the cytokine to form ternary signaling complex and control inflammation.

The IL-6 - olokizumab complex was visually inspected using PyMOL and LigPlot+. The epitope and paratope amino acids were mapped through DiscoTope, Paratome server, respectively. From the potential twenty seven paratope amino acids of the Ag-Ab interacting site, four amino acids of olokizumab (Tyr56, Tyr103 in heavy chain and Gly30, Ile31 in light chain) are selected for site directed mutagenesis with Ser, Thr, Tyr, Trp, and Phe. From the set of 899 theoretical antibodies generated, eight antibodies (Figure 1) have revealed better binding affinity compared to olokizumab in three protein-protein docking servers (ZDOCK, ClusPro and RosettaDock)

as well as binding free energy and interaction energy calculations (CHARMm). Therefore, these eight antibodies (Figure 2) were proposed as potential lead antibodies for neutralization of IL-6 subject to further *in vitro* and *in vivo* evaluations.

This study has noted that the three docking servers effectively predicted native binding orientations of IL-6-olokizumab complex. Therefore, ZDOCK, ClusPro and RosettaDock inspire confidence for being used for antigen-antibody docking/protein-protein docking to predict binding orientations as well as affinities. In addition, the eight proposed potential lead antibodies opens a new avenue for further refinement in the existing therapeutic antibodies for future development of biologics against cytokine-mediated autoimmune and inflammatory diseases by breaking the general norms of searching novel targets and therapeutic molecules. Moreover, the *in silico* protocol adopted in our work may be used as starting steps in antibody designing experiments.
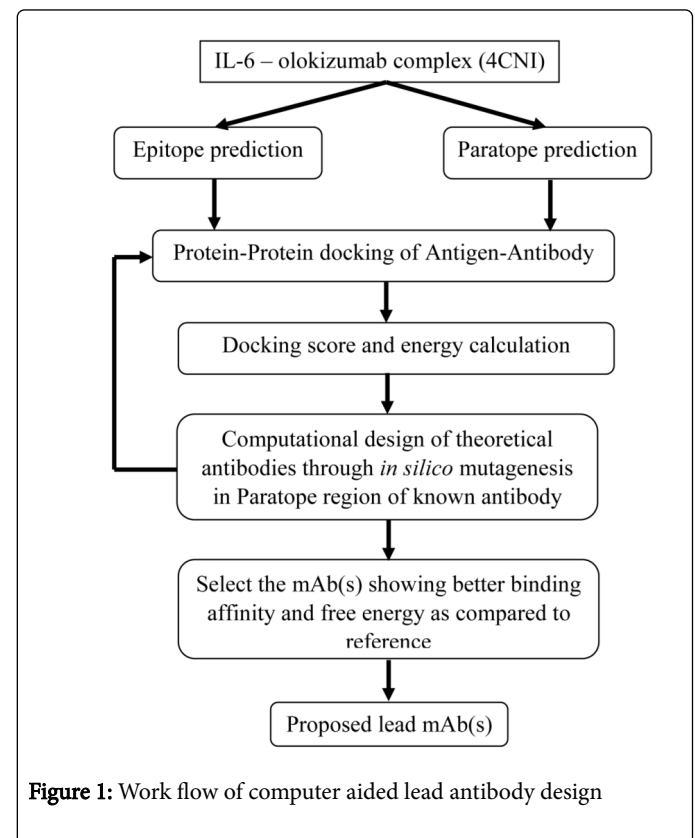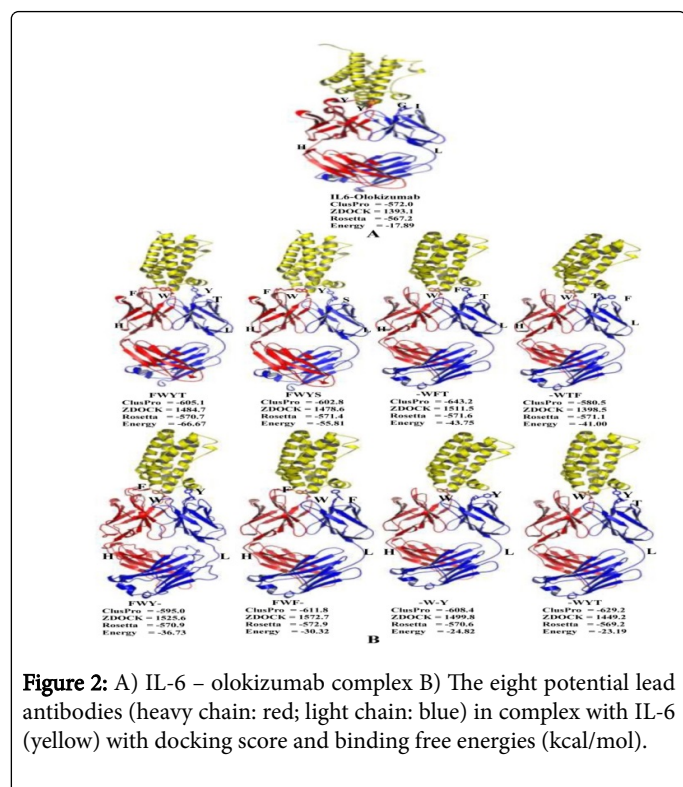

Figure 1: Work flow of computer aided lead antibody design

Figure 2: A) IL-6 – olokizumab complex B) The eight potential lead antibodies (heavy chain: red; light chain: blue) in complex with IL-6 (yellow) with docking score and binding free energies (kcal/mol).

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