## SUMMARY AND CONCLUSIONS

Driven by queries on presence of any shape difference between neutralizing and non-neutralizing IgG1 mAbs reactive to same ligand, we studied some mAbs in this work. Our shape analysis experiments revealed that: 1) Fab arms of the non-neutralizing mAbs are extended away, while the Fab arms in the neutralizing mAbs are not extended away in space probably due to Fab/Fc or Fab/Fab interactions, which remain so even after excision of the extended Fab by limited proteolysis, and 2) neutralizing mAbs can bind only one ligand (gp120, in our case), while non-neutralizing mAbs can bind up to two ligands. The unextended Fab arms were also seen in delay in papain digestion of the Fab arms in case of neutralizing mAbs. We also found that Fab-Fc linker from partial digestion of neutralizing mAbs adopt a L-shape as seen for the closed Fab arm of b12 antibody in the crystal structure of full-length mAb. Surprisingly, our ELISA results confirmed that the Fab-Fc fragments were unable to bind its ligand or C1q. Neutralizing mAbs that bind to one ligand only (gp120 or gp41), also binds firmly to C1q molecule , and the value increased after first binding mAbs to their ligands and then to C1q. This suggested that both ligand as well as facilitation of C1q binding is done by the extended arm of the mAbs, and *partial rigidity induced in that arm due to twisted Fc or the locking in of the closed arm*, probably gives a conformation to the hinge of the extended arm which is recognized by C1q. Relatively reduced binding potency of non-neutralizing mAbs and the extended Fab arms in their global shape suggests that the varied conformations accessible to their Fab-Fc hinges/linkers do not allow effective binding of C1q. Alternatively, it has been shown that by introducing rigidity in the linker region, researchers could induce neutralizing potency in IgG3 mAbs (Lu, Harding et al. 2007).

So while, it is apparent that extended arm has a role to play in ligand and C1q binding, it remains unclear the role of the closed Fab arm besides contributing indirectly to non-flexibility of the extended Fab arm. It is well accepted that during normal immune response antibodies are selected for their ability to bind to foreign antigen with high affinity facilitated by their ability to undergo homotypic binding, but this binding is not always possible. For these characteristics to be effective, the target epitopes must be spaced closely together on the surface of the viral or bacterial pathogen. It has been noticed that over decades, the HIV is continuously reducing the number of trimeric spikes of gp120/gp41 on its surface, key molecule for entering host CD4+ cells. The small number of gp120 glycoprotein spikes displayed on the surface of the human

immunodeficiency virus (HIV) may not favor homotypic bivalent antibody binding (Zhu, Liu et al. 2006; Liu, Bartesaghi et al. 2008; Klein, Gnanapragasam et al. 2009). Thus it can be speculated that whereas antibody evolved to form a bivalent structure that enhances binding to pathogen surface through avidity effect, HIV evolved a low spike density to specifically to thwart bivalent binding by antibodies spike density (Klein and Bjorkman 2010). To counter, antibody too developed polyreactivity to tackle presence of low spike density of HIV-1. Polyreactive antibodies are capable of bivalent hetroligation between one high-affinity anti-HIV-gp120/41 combining site and a second low-affinity site on another molecular structure on HIV like membrane *etc* (Mouquet, Scheid et al. 2010). Although cross-reactivity to self-antigens or polyreactivity is strongly selected against during B-cell development, it is a common serologic feature of certain infections in humans, including HIV, Epstein-Barr virus and hepatitis C virus (Haynes, Fleming et al. 2005; Mouquet, Scheid et al. 2010). Despite the low affinity of the polyreactive combining site, hetroligation or ligand promiscuity demonstrably increases the apparent affinity of polyreactive antibodies to HIV-1. Overall, ours is the first report providing insight into differences in the global shape of neutralizing *vs.* non-neutralizing mAbs. Future studies will keep exploring whether other neutralizing and non-neutralizing mAbs reactive to HIV components also have the characteristics described by us, and whether neutralizing mAbs against other antigenic components also have closed Fab arms. It is very important to mention here that though SAXS based shape characterization would be very useful, papain based digestions too may provide insight.

Based on the speculations made by others and observation made by our group, we propose

a) the structure of intact neutralizing and non neutralizing mAbs (**Figure 6.1**)

b) the binding pattern for C1q to nmAbs and non nmAbs (**Figure 6.2**)