self-associates to a greater extent than M7 and M10 under these conditions. Replacement of the mAb1 charged residues in both the VL and VH chain resulted in a lower molecular weight, and was similar to the M10 mutant. Thus, the self-association behavior of mutant M10 where residues were substituted into the mAb2 structure is consistent with the observed low viscosity–concentration profile. The question, however, still remains as to why the charged residue substitutions into mAb2 did not result in increased self-association and increased viscosity.

Coarse-grained molecular dynamics computations

Molecular dynamics simulations have been done on a full-length mAb and proven useful in understanding how the Fc and Fab domains move and interact with each other (Brandt, Patapoff, & Aragon, 2010). Although with increases in computational power where it is possible to simulate the motion of all the atoms in a single molecule the size of an IgG_1 mAb, it remains a formidable problem to do the simulations for a large number of mAbs in solution. Given the interest in understanding how the mAbs are self-associating to form clusters or networks over relatively long time and trajectories requires an alternative approach. Coarse-grained (CG) modeling, where sites are constructed with appropriate physical parameters allow for the simulation with many molecules (Izvekov & Voth, 2005; Voth, 2009). Representative CG models were generated to specifically investigate the formation of clusters in mAb1 and mAb2 (Chaudhri et al., 2012). The model used included two with a compact configuration (Fab–Fab angle of 36°) and an extended configuration (Fab–Fab angle of 130°) to evaluate the

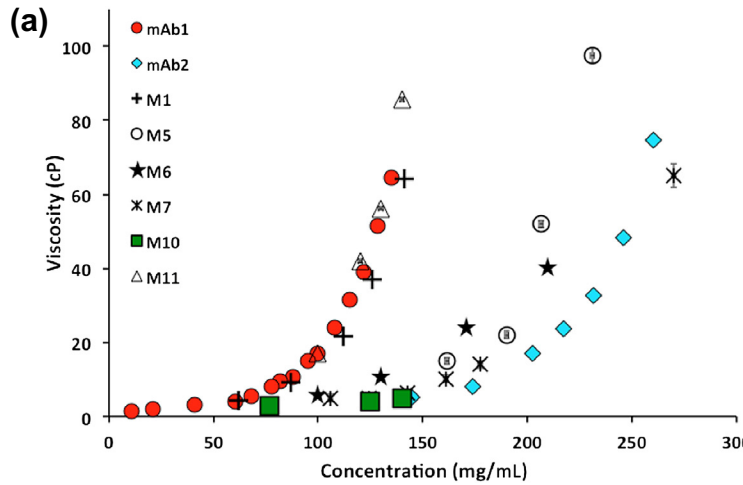

Figure 9.12 (a) Viscosity as a function of mAb concentration of mAb1 and mAb2 mutants with (b) schematics showing amino acid substitutions. All mAbs and mutants are at pH 6 and 15 mM ionic strength. Viscosity determined using a cone-plate rheometer at a shear rate of 1000/s at 25 °C. Adapted from figures created by Sandeep Yadav.