column the net negatively charged protein would elute after the positively charged protein. However, localized concentrated regions of net negative or positive charge may influence the order of elution. In addition to this, protein surfaces are irregular resulting in different exposures of charged groups to solvent. Thus, the flexibility of the protein will also dictate the order of elution since in some cases a fair amount of structural distortion must occur to maximize charge–charge interactions at a substantial cost of free energy. Ion exchangers using extended linkers to place charges on the matrix have been developed (Figure 2.1; Muller, 1990). The extended linkers were generated by chemical modification where polymerized acrylamide derivatives were grafted onto hydrophilic supports. Chromatography using these types of matrices has been termed tentacle ion exchange chromatography and theoretically will enable the protein-charged residues to interact with the matrix without large distortion of protein

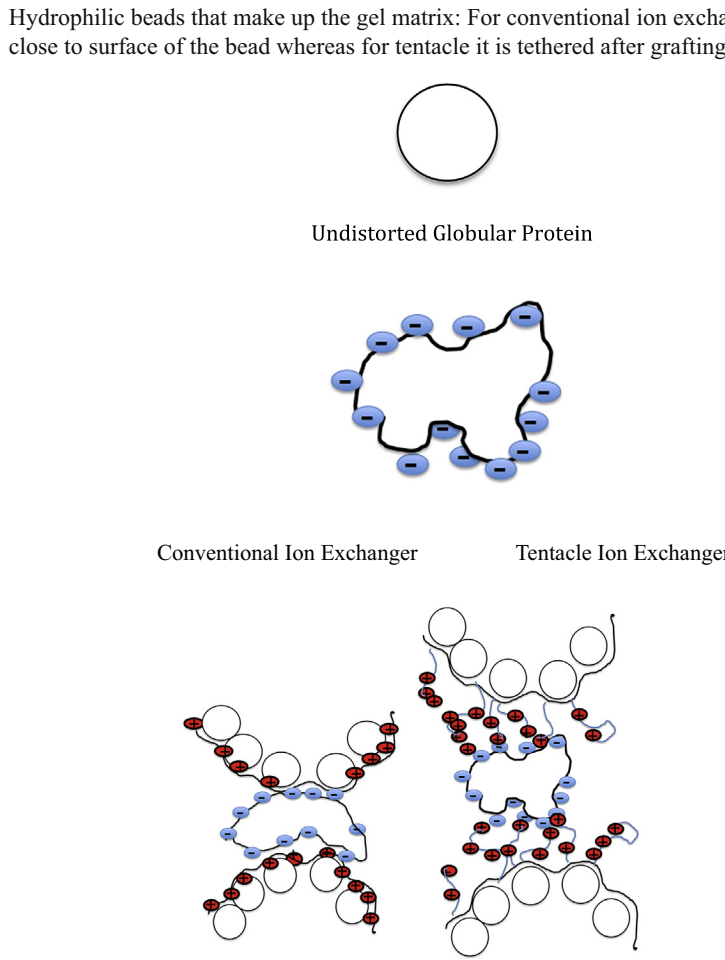

Figure 2.1 Schematic of tentacle ion exchangers compared to conventional ion exchangers (shown for an anion exchange resin).