structure (Donovan, Rabel, & Zahran, 1991; Weitzhandler et al., 1998). Antibodies with flexibility at the hinge region may accommodate some of the distortion, and essentially the mAb structure can distort to maximize the interaction of the surface charged moieties on conventional ion exchangers with the mAb charges. Several studies using tentacle ion exchange resins for mAb purification have been published and involve exploring different resins, which have different support matrices and density of tethered charges. The main point of these investigations was to increase recovery capacity when compared to conventional protein-A chromatographic media. Tentacle cation ion exchange chromatography was used during development of Pulmozyme® (an inhaled recombinant derived human DNase for treatment of cystic fibrosis) to attempt to improve the ion exchange resolution (Cacia, Quan, Vasser, Sliwkowski, & Frenz, 1993). Surprisingly, only two peaks were resolved and it was then determined that the tethered negative charges mimicked the poly PO_4 backbone of DNA which bound specifically to a site with an exposed Asn residue. Deamidation of the residue weakened this interaction resulting in two peaks, one the deamidated and the other the non-deamidated form of Pulmozyme®. This was nicely shown using isoelectric focusing (see details for this technique below) where each resolved peak had several charged species of either the deamidated or non-deamidated Pulmozyme® (see Figure 7 of Cacia et al., 1993).

Hydrophobic interaction chromatography

Hydrophobic interaction chromatography (HIC) separates proteins based on the hydrophobicity of the protein (Queiroz, Tomaz, & Cabral, 2001), and has been used to follow oxidation and Asp isomerization. Hydrophobic interactions tend to be strongest at high salt concentrations. Small hydrophobic residues such as phenyl or propyl groups are coupled to the chromatographic matrix. After loading in high salt the protein is eluted using a decreasing salt gradient. In this manner the more hydrophobic proteins elute later during the chromatography. This type of chromatography was successfully used to separate four different forms of tissue plasminogen activator (tPA) (Wu, 1992). It has been very useful in analyzing mAbs for Asp isomerization and oxidation of Trp and Met (Boyd, Kaschak, & Yan, 2011; Valliere-Douglass, Wallace, & Balland, 2008). Asp isomerization in the complementarity region of a mAb that binds to IgE was further elucidated on HIC by using either pepsin to generate $F(ab)'_2$ fragments or papain to generate Fab and Fc fragments via cleavage at the mAb hinge region (Cacia, Keck, Presta, & Frenz, 1996).

Reversed phase chromatography

Reversed phase chromatography (RP-HPLC) uses resins with small hydrophobic groups attached. Instead of using salt gradients to elute hydrophobic species, organic modifiers such as acetonitrile or propanol are added to the elution buffer to decrease the water concentration in the mobile phase. This in turn weakens the hydrophobic attraction of the hydrophobic groups on the chromatography matrix for the protein. This is akin to what happens in the folding of proteins in aqueous media. The hydrophobic forces are due to structuring of water around the hydrophobic residue resulting