Chapter Six: Conclusions and Recommendations

This work has evaluated the role of local density effects upon two classes of affinity adsorbents. The problem was divided into three phenomena that were uncoupled by the combination of the use of a low solids content cellulose matrix having rapid mass transport of the target protein and the spatial manipulation of matrix activation. The immobilization of monoclonal antibodies in one case and peptide ligands enabled the relative evaluation of the above phenomena for immunoaffinity and affinity matrices. In spite of the great molecular size differences between antibodies and peptide ligands, the role of local density of the immobilized ligand was shown to be significant for both classes of affinity sorbents.

The distribution of either ligand type within the support particle was shown to impact target binding efficiency. The highest binding efficiency was shown in those matrices having the lowest local density at any given support average density. This may be attributable to a decrease in steric hindrance effects and therefore an increase in the accessibility of the immobilized ligand towards the protein target. This work represents the first time that either monoclonal antibodies or peptide affinity ligands were immobilized using a pre-established gradient of activation sites within the matrix so that covalently coupled ligands with a pre-determined local density might be achieved. However, we did not conduct labeling experiments of either target or immobilized ligand which could simultaneously quantify both ligand binding efficiency and local immobilized density of the ligand. Here, the local density of matrix activation was used as an indirect indicator of the local ligand density. Specifically, a fluorescent molecule was used to terminate all of the activation sites in a parallel treatment of a matrix sample aliquot from the same reaction mass product used in the ligand immobilization experiments. The gradient of activation sites was clearly shown to be less dense at the edge and increasingly more dense in the interior of the cellulose matrix.
Orientation effects were also found to be important to the activity of affinity peptide sorbents as has also been shown in prior studies with immunosorbents. The N-terminal amino acid residue of the recognition sequence of the affinity peptide ligands used here were required to be free (unmodified) in order to effectively form a complex with the target protein. For example, the affinity sorbents prepared using peptide ligands immobilized through their C-terminal glycine residue, as opposed through their N-terminal amino acid residue, were found to exhibit higher binding efficiency. The use of a carbodiimide-mediated coupling chemistry enabled the cross-linking (polymerization) of multiple peptide ligands, which was also attributed to increasing the binding efficiency of the affinity sorbents due to increasing the accessibility of the recognition sequence to the target protein. The use of a branched peptide ligand structural format containing multiple recognition sequences was also found to increase binding efficiency by increasing the availability of the number of recognition sequences contained in each ligand capable of forming the binding interactions with the target protein.

Our studies were focused on the effects of achieving a gradation of spatial distribution of activation, which would result in a gradation of immobilized ligand. However, we did not optimize ligand coupling yields, ligand loadings and ligand orientation with respect to the overall economy of a general technique for ligand immobilization and affinity adsorption efficiency. For example, solution phase polymerization of the ligand was too extensive under our experimental conditions resulting in the loss of peptide or ligand available for immobilization and thus low yields. The use of masking agents, such as dimethylmaleic anhydride or the target protein itself, may aid in preventing or minimizing the crosslinking of the peptide ligands during carboxy- or amino-activation of the ligand prior to immobilization. This reversible capping chemistry could therefore increase the amount of ligand directly immobilized onto the support. In addition, these
masking agents may further aid in spatially orienting the immobilized ligand within the support in an optimal manner for more effective target protein binding.

The pre-activation of the ligands with compounds, which also serve as linkers, may further serve to increase binding efficiency by extending the ligand away from the support. Our work undoubtedly and indirectly caused some crosslinking which would serve in some case for creating a linker for immobilization of a portion of the ligands that were immobilized. More preferred would be the use of linkers specifically designed for that purpose. These linkers would have more easily managed reactivity with the ligands. This could be achieved either through solution phase coupling to the ligand or attachment to the matrix prior to ligand attachment. The use of a linker would permit more degrees of freedom in the orientation of the immobilized ligand and thereby decrease conformational restrictions. A less conformationally restricted immobilized ligand would more readily form a complex with the target protein.

Transport phenomena and the spatial distribution of immobilized ligand were also found to affect immunosorbent and peptide-based affinity sorbent performance. The use of a low-solids content, large-particle diameter cellulose support was shown to be an effective platform in the chromatographic isolation of high-molecular weight proteins. However, extensive determination of the spatial distribution of immobilized ligands was not studied. Chromophore or otherwise detectable ligands that are labeled should be used to quantify the spatial distribution. Since some affinity peptides may demonstrate cooperative binding properties, the spatial distribution and local density of the immobilized affinity peptide ligands should be studied to determine an optimal immobilization procedure to maximize binding efficiency. The use of a fluorescent compound, similar to fluorescein isothiocyanate, coupled to the peptide ligand would enable the spatial position and local density of the immobilized peptide ligand to be studied. However, incorporating a fluorescent compound may annihilate or
substantially decrease the ability of the peptide ligand, which is typically of low molecular weight and size, to couple to the support. The decrease in reactivity of a labeled peptide ligand may be attributed to steric hindrance in which the reactive functional groups on the peptide ligand serving to form the covalent linkage with the activated support are inaccessible. The florescent label would have to be attached to the peptide ligand in a manner so as not to interfere with the formation of the covalent linkage with the support. Additionally, the coupling reaction chemistry and conditions used may also adversely affect the florescent label compound.