535d Orientational Control of Immobilized Biomolecules on a Membrane Surface for Specific Protein Capture

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Track-etched membranes, arranged in a stack, have recently found application in high- throughput analytical techniques for protein expression profiling. Examples include sampling blood serum for antibodies or other proteins that indicate disease and blotting tissue sections for specific biomarkers that may be unique to tumor cells. If maintaining sample morphology is important (as in tissue sections), track-etched membranes offer an additional advantage over conventional blotting membranes by minimizing image distortion due to lateral diffusion. The use of layered membranes produces replicates of each sample from a single transfer. Each membrane is then treated with a specific antibody for detection of a particular protein of interest. However, since proteins are depleted as they pass through the stack, a result of nonspecific binding, only a limited number of proteins can be analyzed and there is a loss in sensitivity. Because of these limitations, there is a need to develop membranes with enhanced specificity for a particular protein with minimal nonspecific binding. While traditional immobilization techniques, such as glutaraldehyde crosslinking, can result in a surface with some bioaffinity, they also cause severe losses in biological activity through denaturation, which in turn can significantly compromise sensitivity towards the target protein.

In this study, two approaches of attaching proteins to the surfaces of track-etched membranes will be presented. The first approach relies on the ability of nickel ions to simultaneously chelate to amine groups on the membrane surface and bind to histidine groups that have been genetically engineered onto the terminal end of a protein to be immobilized. The second approach is based on the enzymatic attachment of a protein through its tyrosine residues to amine groups on a membrane surface. In both cases, the immobilization techniques resulted in improved control over the orientation of biomolecules on the membrane surface. This was demonstrated by measuring considerable enhancements in the levels at which target proteins could be detected. The results were compared to those using conventional crosslinking techniques. Single-site Langmuir isotherms were used to describe the binding of the target protein to the membrane surface.