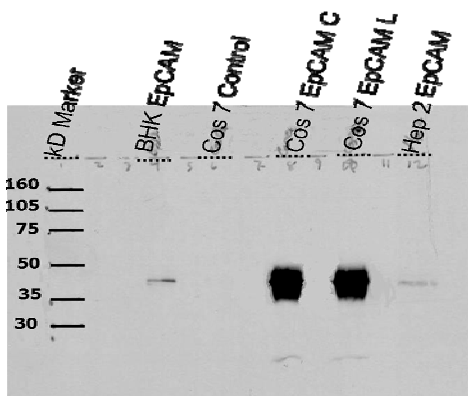
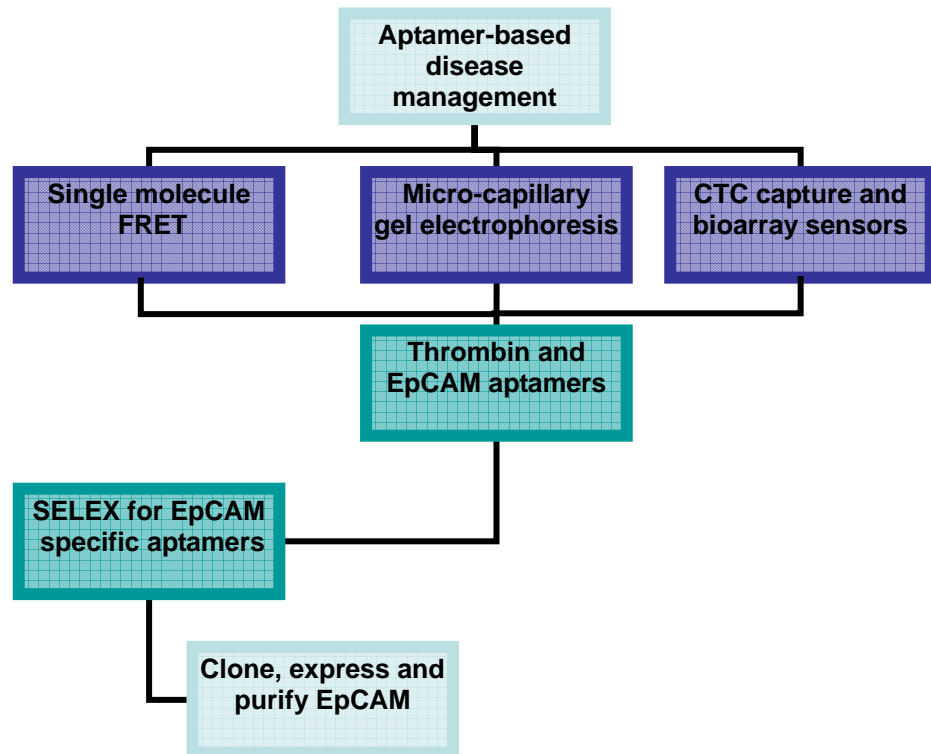


## Development and utilization of aptamers as affinity probes for disease management

Aptamers have emerged as alternative affinity agents for utilization in bioanalytical techniques. These single stranded oligonucleotides with comparably high affinity for their targets as monoclonal antibodies, are more chemically stable and do not denature on immobilization to surfaces as is observed with proteins. This makes them attractive for incorporation in analytical devices. Aptamers are selected toward a target by Systematic Evolution of Ligands by EXponential

enrichment (SELEX). Our research focuses on utilization of aptamers as affinity probes for disease management as depicted in the flowchart.



Aptamer selection against targets, such as proteins, requires acquisition of the analyte in the purest form possible. We selected the epithelial adhesion molecule (EpCAM) which is over expressed in breast cancer as a target for aptamer selection. Our work involves cloning of EpCAM into mammalian expression vectors for producing a histidine tagged variant of the

extracellular and transmembrane portions of this biomarker in high yield and

easily purified form for SELEX. Using two different cloning vectors, the TA cloning system and an episomal expression vector, three different mammalian cell types were transfected. Western blot results indicate that the episomally expressing Cos 7 cells were more stable and produced the highest yield of recombinant EpCAM. Thus, transfected Cos 7 cells were selected for large scale EpCAM expression and purification.

Aptamers can find use in various analytical techniques for quantification of biomarkers or capture and release of targets. To study the interaction of surface immobilized aptamers with target analytes on gold and poly(methylmethacrylate), PMMA, we utilize techniques such as laser scanning confocal microscopy (LSCM) and surface plasmon resonance (SPR) spectroscopy. Other aspects of our research include surface modification of polymer-based microfluidic devices, through UV oxidation and carbodiimide coupling of aptamers into the microchannels for cell capture studies. We are also involved in utilizing microchip capillary electrophoresis with fluorescence detection and single molecule FRET for quantifying thrombin in real samples.