

## Design of Biomimetic Microspheres for Biosensing in Packed Microcolumns

G.P. Lopez

Center for Biomedical Engineering and Department of Chemical and Nuclear Engineering,  
University of New Mexico, Albuquerque, NM 87106, U.S.A.

**INTRODUCTION:** This talk will present recent developments at the University of New Mexico in the analysis of biomolecular recognition in microfluidic systems. The method involves real-time detection of soluble molecules binding to receptor-bearing microspheres, sequestered in affinity column-format inside a microfluidic channel. The packed microcolumn format is (1) well suited for enhancing reaction times of analyte with immobilized receptors, (2) compatible with electro-osmotic pumping, and (3) allows detection of multiple analytes. Identification and quantitation of analytes occurs via direct fluorescence measurements or fluorescence resonance energy transfer (FRET). Several immunoassays have been developed that can potentially detect sub-femtomole quantities of antibody with high signal-to-noise ratio and a large dynamic range spanning nearly four orders of magnitude in analyte concentration in microliter to submicroliter volumes of analyte fluid.

**METHODS:** Surface modified microbeads (e.g., either silica beads or commercial streptavidin coated beads) are sequestered into microfluidic channels formed by soft lithography or fused silica capillaries to form a packed microcolumns. Sample injection into the columns and subsequent analyte reactions are detected by fluorescence spectroscopy.

**RESULTS:** Figure 1 shows examples of the packed microcolumns. In this Figure data obtained from a microcolumn containing three distinct sensor regions are presented. A number of different types of biomolecular interactions can be investigated in this type of column. For example, selective binding of antigens to surface immobilized antibodies can be detected directly by monitoring FRET between dyes conjugated to the respective immunological reagents.

Kinetic and equilibrium constants for the reaction of receptor-ligand pairs can be obtained through modelling of kinetic responses of the affinity microcolumn and are consistent with those obtained by flow cytometry. Because of the correlation between kinetic and equilibrium data obtained for the microcolumns, quantitative analysis can be done prior to the steady state endpoint of the recognition reaction.

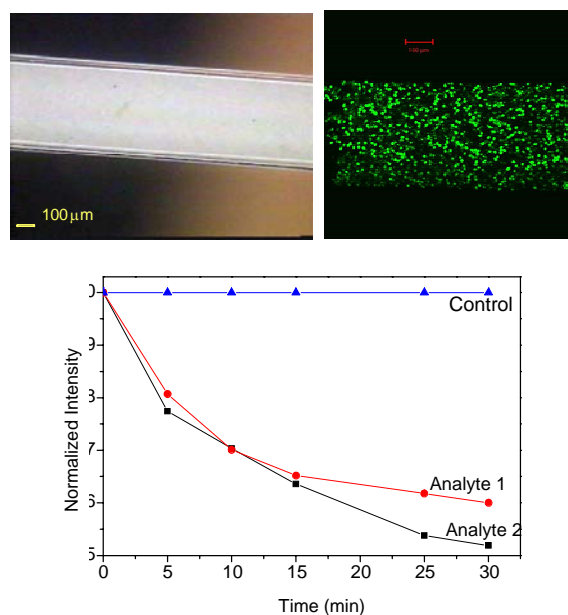


Fig. 1: Top: Optical and fluorescence micrographs of packed microcolumns. Bottom: Typical response of multianalyte microcolumn to injection of equal amounts of two analytes.

**DISCUSSION & CONCLUSIONS:** This method has the promise of combining the utility of affinity chromatography, with the advantage of direct, quantitative, and real-time analysis and the cost-effectiveness of microanalytical devices. The approach has the potential to be generalized for high sensitivity, high selectivity, rapid detection of a host of bioaffinity assay methods and analyte types.

**REFERENCES:** "Near Simultaneous and Real Time Detection of Multiple Analytes in Affinity Microcolumns," Piyasena, M.E.; Buranda, T.; Wu, Y.; Huang, J.; Sklar, L.A.; Lopez, G.P. *Anal. Chem.* **2004**, *76*, 6266-73. "Biomimetic Molecular Assemblies on Glass and Mesoporous Microbeads for Biotechnology," Buranda, T.; Huang, J.; Ramarao, G.V.; Ista, L.K.; Larson, R.S.; Ward, T.L.; Sklar, L.A.; Lopez, G.P. *Langmuir* **2003**, *19*, 1654-1663.

**ACKNOWLEDGEMENTS:** This work was supported by the National Science Foundation (CTS032315).