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Surface immobilization of peptides on SiO2 nanobeads for an enzymatic biosensor

We have been investigating catalytic systems for the development of a biosensor that detects medically relevant enzymes. Two peptide sequences, CF6 and 051-4 were examined as a trypsin substrate. The peptides were synthesized with two fluorophores, AMCA (donor) and FITC (acceptor), attached to either ends of the peptides to allow for fluorescence resonance energy transfer (FRET) sensing. Sensors utilizing FRET switch their fluorescence wavelength between the donor and the acceptor dyes as distance between the two dyes change. When the peptide is cleaved by trypsin, the donor and acceptor fluorophores are separated, resulting in a detectable change in fluorescence. The peptides were immobilized onto silica nanobeads using two different techniques: silanization and simple adsorption. Nanobeads were utilized in order to increase the surface area for the peptide immobilization. The substrates were then exposed to trypsin. The results showed that the silanization method had increase in binding capability than simple adsorption. The adsorption method had poor initial fluorophore signal with little response to trypsin, most likely due to de-adsorption during the assay. The silanization method showed excellent dosage response with a limit of detection at 0.0001% trypsin.