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Enhanced antibody immobilization on magnetic silica nanoparticles using multimeric protein Gs

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Abstract:

This talk will describes a method for the effective and self-oriented immobilization of antibodies on magnetic silica-nanoparticles using a multimeric protein G. Cysteine-tagged recombinant dimers and trimers of protein G were produced in *Escherichia coli* BL21 by repeated linking of protein G monomers with a flexible $(GGGGS)_3$ linker. Amino-functionalized silica-coated magnetic nanoparticles (SiO_2-MNPs, Fe_3O_4@SiO_2) were prepared and coupled to the protein G multimers, giving the final magnetic immunosensor. The optimal conditions for the reaction between the protein Gs and the SiO_2-MNPs was a time of 60 min and a concentration of 100 g/mL, resulting in coupling efficiencies of 77%, 67% and 55% for the monomeric, dimeric and trimeric protein Gs, respectively. Subsequently, anti-hepatitis B surface antigen (HBsAg) was immobilized onto protein G-coupled SiO_2-MNPs. The quantitative efficiency of antibody immobilization found the trimeric protein G to be the best, followed by the dimeric and monomeric proteins, which differs from the coupling efficiencies. Using all three protein constructs in an HBsAg fluoroimmunoassay, the lowest detectable concentrations were 500, 250 and 50 ng/mL for the monomeric, dimeric and trimeric protein G-coupled SiO_2-MNPs, respectively. Therefore, multimeric protein Gs, particularly the trimeric form, can be employed to improve antibody immobilization and, ultimately, enhance the sensitivity of immunoassays. In addition, the multimeric protein Gs devised in this study can be utilized in other immunosensors to bind the antibodies at a high efficiency and in the proper orientation.