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Novel DNP-KLH Protein Conjugate Surface for Sensitive Detection of TNT on SPR Immunosensor

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A nanoscale biosensor chip surface for the detection of trinitrotoluene (TNT) was fabricated using dinitrophenylated-keyhole lympet hemocyanin (DNP-KLH) protein conjugate as ligand supported by an underlying 11-amino 1-undecanethiol hydrochloride (AUT) self-assembled monolayer (SAM) and bis sulfo-succinimidyl suberate (BS^3) as crosslinker. Bioactive thin films were fabricated over gold chip via layer-by-layer self-assembly methods. The biomolecular interaction between substrate-specific TNP-glycine-KLH mouse Ig antibody and DNP-KLH conjugate surface was monitored using a surface plasmon resonance-based optical sensor. The quantitation of (TNT) on this bioactive surface was carried out using the solution-based competitive inhibition assay. The DNP-KLH surface biosensor has shown a detection limit of 0.15 ng/ml (150 ppt) and was sensitive up to the 0.005 ng/ml (5 ppt) level for the TNT molecule. This TNT-specific biosensor holds the promise to be one of the most sensitive, fast TNT detecting sensor surfaces under indirect competitive assay format. A 12 s injection pulse of 10 mM glycine-HCl solution was sufficient for the regeneration of the DNP-KLH surface for repeated use. The DNP-KLH sensor platform was checked for its reproducibility and storability.

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