Covalent Functionalizing Transition Metal Dichalcogenides with Aptamers for Biosensing

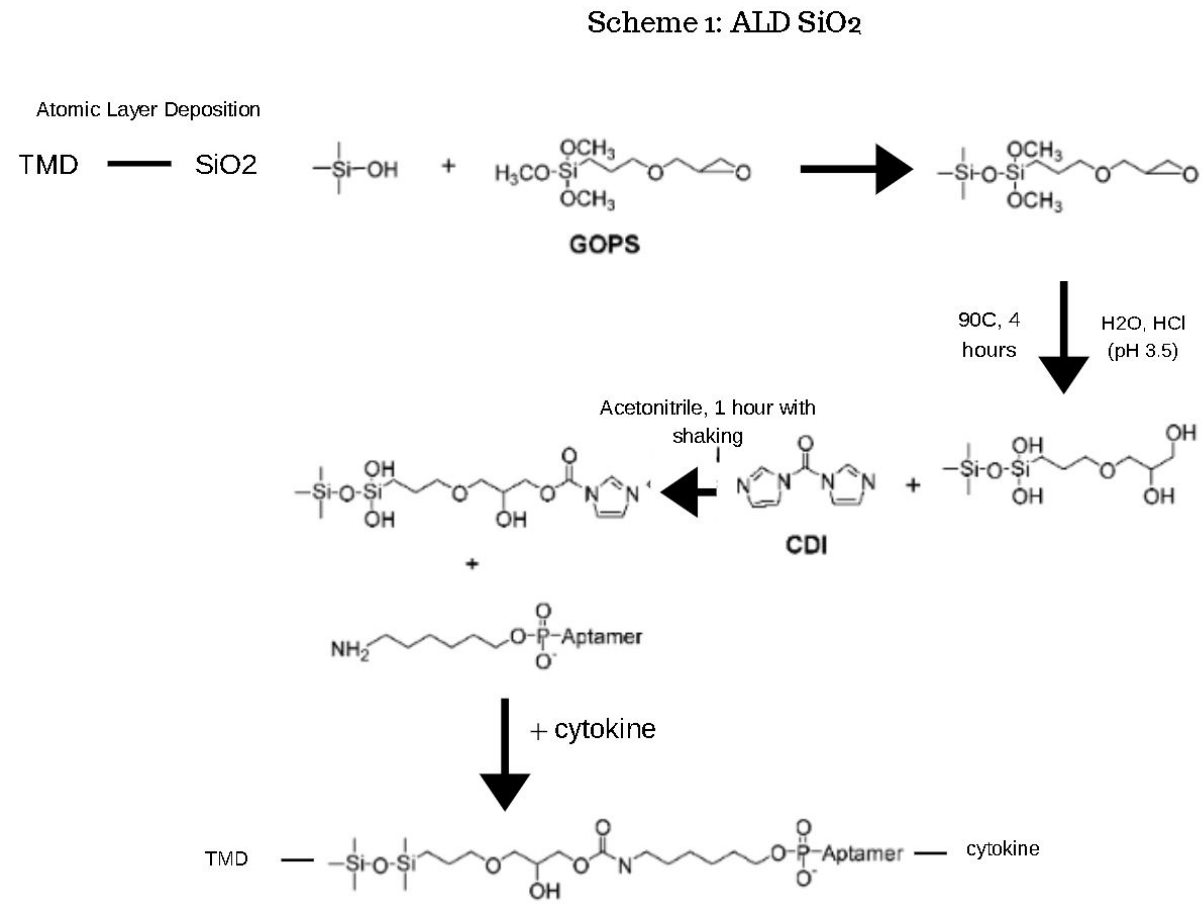
The focus of this research is to create a modular method that covalently functionalizes transition metal dichalcogenide(TMD) surfaces with analyte targeting aptamers to selectively bind with cytokines: Interferon gamma (IFNg) and Tumour Necrosis Factor alpha (TNFa). Aptamers are synthetic chains of nucleotides made using in vitro selection (SELEX) through repeated purification and washing cycles. Aptamer functionalized TMD (ft-TMD) are generalizable for a range of analytes and applications include target therapy, acoustic wave, fluorescent, and electrochemical biosensors.

Cytokines are inflammatory proteins in our body that are related to a variety of diseases including cancer, HIV, Atherosclerosis, and Alzheimer's. Detecting such proteins are useful for early disease diagnosis and could be used to measure the effectiveness of new treatments.

Two proposed functionalization methods are: defect engineering and silane/ peptide functionalization. For the first method, sulfur vacancies defects in TMDs are filled by covalent attachment of thiol terminated aptamers. The second method involves atomic layer deposition on TMD a monolayer of silica, which is the base for silane chemistry with linkers to form a peptide bond with amine terminated aptamers as shown in Scheme 1.

TMDs are piezoelectric material which has changing electrical properties in response to mechanical stress and vice versa. Therefore, TMDs are used to build highly sensitive acoustic wave sensors which will change its resonant frequency in response to varying masses bound to its surface. Thus using this method, we can attach aptamers tuned to bind with specific molecules with TMD therefore allowing us to accurately detect target molecules. Another target-reporting mechanism is the fluorescent sensing, which utilizes dye-labeled aptamers covalently coupled onto the TMD surface. The changing fluorescent signal indicates binding with the target cytokine. This is done by extending the original aptamer sequence with complementary necleotides of the target capturing nucleotides. Because of the complementary-base pairing, the extended aptamer loops back and brings the dye close to the TMD's surface shown in Figure 1. TMDs will quench the, now close, dye via FRET quenching. When the aptamers' target binding sequence binds to the target cytokines, the tertiary shape of the aptamers will change to conform to the target. The shape change moves the fluorescent molecule away from the TMD surface and once again fluoresces as depicted in Figure 1. The fluorescence change indicates bonding with target cytokines. An example of successful covalent functionalization of thiolated fluorescent labeled aptamer on TMDs is shown in Figure 2.

TMDs covalently functionalized with aptamers are reusable and more robust than the current standard: single-use ELISA antibodies immunoassays. The proposed covalent functionalization of TMD will allow target molecules to be washed off from the sensing layer without damaging the TMD sensing substrate for subsequent sensing. In addition to its reusability, the success of this project would reduce the ELISA clinical standard of 4+ hours in cytokine detection, save on expenses in bulky plate reader equipment, chemicals, and sophisticated ELISA skillsets.



Scheme 1: Method of covalently binding amine terminated aptamer to TMD

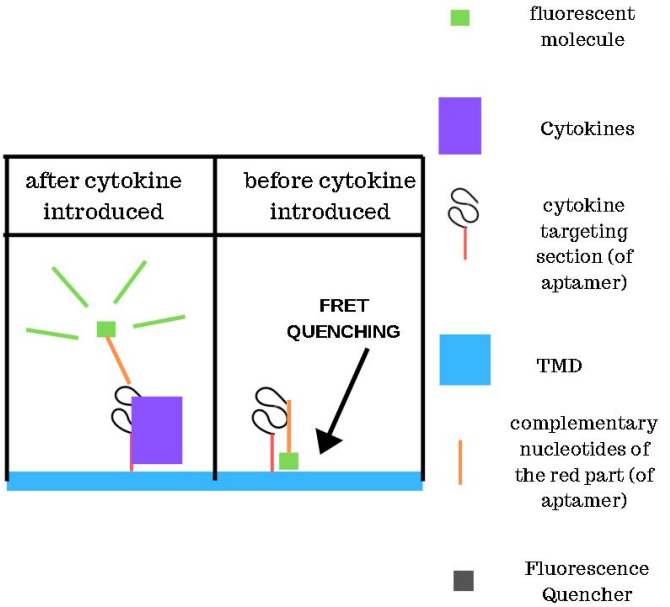


Figure 1: Scheme for fluorescent biosensor design: Complementary Loopback Fluorescent Aptamer

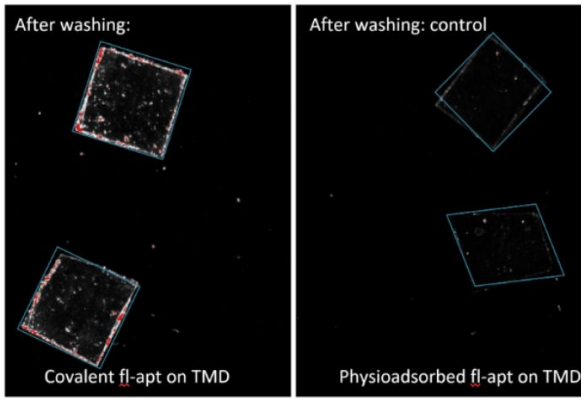


Figure 2: Fluorescence images of covalently functionalized vs. physioadsorbed dyed-aptamer on TMD (Thiol method)