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Biosensor surface attachment of the ovarian cancer biomarker HSP10 via His-tag modification

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ABSTRACT

Herein, we describe a new protocol for the silane-based surface modification of quartz substrates for the immobilization of hexa-histidine-tagged heat shock protein 10. This molecule is an attractive biomarker for the detection of ovarian cancer, particularly for possible early-stage diagnosis. The surface chemistry we have described is used for a study of the interaction between the protein and a novel DNA aptamer via an in-house built acoustic wave sensing platform. Quartz discs were modified with a mixed trichlorosilane adlayer and functionalized with a nickel-NTAL moiety to allow for immobilization of the protein in a correctly-oriented fashion. All layers were characterized by x-ray photoelectron spectroscopy to show successful layer modification. Detection of binding in phosphate buffered saline was determined using the EMPAS, operating at a fundamental frequency of 940 MHz. Results regarding the specific binding of the DNA aptamer to the surface-immobilized protein exhibited a significantly smaller signal compared to surfaces without protein, which comparatively yielded much larger frequency shifts. The smaller shift in frequency is attributed to the rigidification of the bulk layer upon interaction between probe and ligand as the overwhelming factor. This rigidification is counteracted by mass loading effects, in which a balance between these two factors is what contributes to the overall smaller frequency shift. Without the binding interaction, the bulk layer does not rigidify, resulting in mass loading being the main contributor to signal, and thus a large frequency shift is seen.

1. Introduction

Ovarian cancer, a deadly disease of the ovaries in the female reproductive system, currently affects approximately a quarter million women worldwide and is anticipated to kill over 14,000 women in the US. Over 22,000 new cases are diagnosed and accounts for the deaths of nearly 150,000 women annually [1,2]. In most cases, the disease is not detected until it has reached its late stages, resulting in the abysmal 5-year survival rate of only 45% compared to the 99% for women with breast cancer [2]. The *only* and current widely used biomarker-based assay for ovarian cancer is detects for the well-known cancer antigen 125 (CA-125) [3–5], a method that has been employed for monitoring patients for over three decades. Unfortunately, the CA-125 assay is not viable on its own for the detection of ovarian cancer, since the antigen is only present at elevated levels in approximately half of patients at the early stage [3]. It is, however, present in over 90% of late-stage patients [6], which is a major disadvantage at a diagnosis and prognosis stand point. Furthermore, the assay is notorious for producing significant amounts of both false negative and false positive results [4,7–9]. In view of the above, there is an urgent need for the development of an assay for a system, especially a biosensor, which is capable of the detection of a biomarker for ovarian cancer in the early stages of progression of the disease. As the first steps towards this goal, we must first evaluate proper probes against a new target protein.

Heat shock protein 10 (HSP10) has recently emerged as a potential biomarker for ovarian cancer [10] as it is seen to promote tumour formation through suppressing apoptosis of malignant cells [11]. The protein has been found to be elevated in concentration in the immediate tissues surrounding a tumour within the ovary [10] and therefore, taking a blood sample from the immediate area would prove acceptable as a possible screening method. HSP10 is not present at elevated levels in healthy individuals, rendering it a plausible biomarker with high specificity and low chance of eliciting false positives [12,13]. It is known that HSP10 is involved in cell proliferation during pregnancy and is found to be released into the bloodstream at these early stages, thus it is also known as Early Pregnancy Factor [10,14]. It is released during pregnancy because HSP10 is also an immunosuppressant [11], thus protecting the fetus from being attacked by the immune system of the mother. As a result, it is difficult to develop antibodies against this protein. In addition to ovarian cancer, HSP10 is also seen to be released from proliferating liver cells during regeneration [12,15] as well as several other cancers [12,16–18]. Therefore, this protein may not need to be

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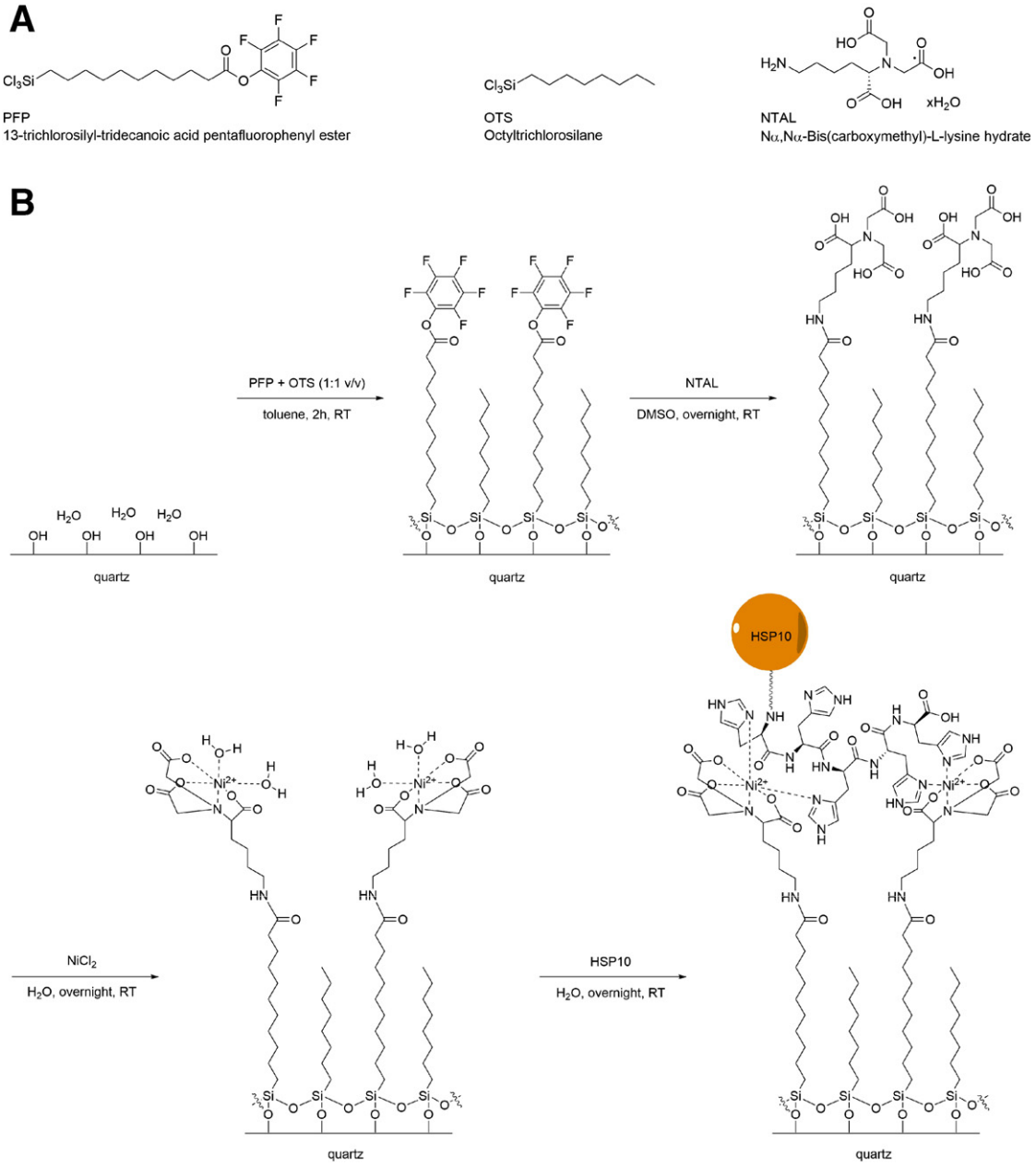
detected specifically for ovarian cancer, but can also be applied to many other disease cases.

In order to design and develop a biosensor for the detection of HSP10, it is necessary to produce a probe capable of highly selective binding of the protein, with the probe being linked to the surface of a device of choice. Among the many molecular recognition entities such as proteins [19,20], antibodies [21] or oligonucleotides [22,23] employed for this purpose, the aptamer offers several desirable properties in terms of the binding of HSP10, especially in the light of the fact that it is extremely difficult to develop antibodies against this protein. Among the oligonucleotide family, since their appearance in the 1990s, aptamers have increasingly become a popular tool for application in novel diagnostic technologies because of their ability to provide high affinity and selective binding towards a very wide range of target analytes [24]. Aptamers are considered to possess significant advantages over the more conventional biological probes such as antibodies and enzymes in that they can be designed and synthesized to be capable of binding to a particular target of interest, are chemically stable, and can be produced on a commercial basis with relative ease [10,11]. An additional attractive feature of aptamer molecules is their capability to undergo characteristic conformational changes, which can be tailored for detection by specific transduction techniques [25,26].

The device of choice in the present work is the electromagnetic piezoelectric acoustic sensor (EMPAS) [27] It couples the advantages of two sensors (thickness shear mode acoustic wave sensor and magnetic acoustic resonance sensor) [28] to achieve extremely high sensitivity. The EMPAS is designed to detect the resonance frequency generated from an electrode-less quartz disc. This resonance is induced by an oscillating electromagnetic field from a copper coil located 30 μm under the quartz disc. Because the EMPAS can generate ultra-high frequencies (up to 1 GHz), it is very sensitive to structural changes, and viscoelastic, and mass loading properties [28]. It is noteworthy also that the device is capable of functioning while operating in biological fluids [29,30].



Scheme 1. (A) Molecular reagents used for surface modification of quartz. (B) Chemical schematic for the surface modification of activated bare quartz to allow for final HSP10 immobilization.