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Development of Surface Chemistry for SPR based Sensors for the Detection of Proteins and DNA molecules

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ABSTRACT

The immobilisation of biological recognition elements onto a sensor chip surface is a crucial step for the construction of biosensors. While some of the optical biosensors utilise silicon dioxide as the sensor surface, most of the biosensor surfaces are coated with metals for transduction of the signal. Biological recognition elements such as proteins can be adsorbed spontaneously on metal or silicon dioxide substrates but this may denature the molecule and can result in either activity reduction or loss. Self assembled monolayers (SAMs) provide an effective method to protect the biological recognition elements from the sensor surface, thereby providing ligand immobilisation that enables the repeated binding and regeneration cycles to be performed without losing the immobilised ligand, as well as additionally helping to minimise non-specific adsorption. Therefore, in this study different surface chemistries were constructed on SPR sensor chips to investigate protein and DNA immobilisation on Au surfaces. A cysteamine surface and 1%, 10% and 100% mercaptoundeconoic acid (MUDA) coatings with or without dendrimer modification were utilised to construct the various sensor surfaces used in this investigation. A higher response was obtained for NeutrAvidin immobilisation on dendrimer modified surfaces compared to MUDA and cysteamine layers, however, protein or DNA capture responses on the immobilised NeutrAvidin did not show a similar higher response when dendrimer modified surfaces were used.

Keywords: biosensor, surface chemistry, SPR, dendrimer, MUDA

1. INTRODUCTION

The manner of surface chemistry used to functionalise a biosensor chip is crucial for obtaining good detection signals from the sensor device. Surface plasmon resonance (SPR), quartz crystal microbalance (QCM) and electrochemical sensor chips are usually covered with a metal layer, usually gold (Au). Direct adsorption of proteins onto metal surfaces may cause the denaturation of the protein molecules and also the blocking of the ligand binding sites due to the random orientation of the proteins on the metal surface. Additionally the weak interaction between the surface and the immobilised protein will result in an unstable sensing surface, making it unsuitable for repeated use [1]. To minimise these problems, the use of covalent immobilization is usually recommended in order to realise a more stable sensing layer covering the metal surface. Achieving this can either be through the use of a self assembled monolayer or by using polymeric surface coatings. These will enable biomolecules to be immobilized covalently to the sensor surface, enabling them to be used repeatedly without loss of activity.

Self-assembled monolayer's (SAMs) are one of the simplest examples of self assembly and can be formed by means of silanes on oxidised and hydroxylated surfaces and by means of thiols on metal surfaces. The thiol molecules used to obtain self assembled monolayers are usually alkanethiols with three parts including: a head group (linking group, namely a S-H group), the backbone or spacer (hydrocarbon main chain), and the terminal specific (active) group also known as the tail group [2]. The sulphur atom is semi-covalently bound to noble metal surface due to the strong affinity of sulphur for these metals and the van der Waals forces between the hydrocarbon chains stabilize the structure and create an ordered monolayer. The terminal group of the molecule determines the surface characteristics of the

formed SAM. For example $–CH_3$ and $–CF_3$ terminal groups create hydrophobic and –COOH, $–NH_2$ or –OH groups yield hydrophilic surfaces. SAMs can be formed on surfaces either by simply immersing the metal substrate in a dilute solution of thiol /silane or alternatively they can be formed by vapour deposition [3]. As soon as the metal substrate is immersed in dilute thiol solution, a disorganised monolayer spontaneously forms and then slowly the molecules are organised due to van der Waals forces. The quality of the assembled monolayer depends on the cleanliness of the metal substrate, the purity of the alkanethiol solutions used and the length and composition of the alkanethiol. The head group of the alkanethiol is critical to obtain a monolayer with the required functionality. Many alkanethiols with different head groups are commercially available for the immobilisation of biological recognition elements on biosensor surfaces such as –COOH, $-NH_2$, -OH, -biotin, and -N-hydroxysuccinimide (NHS).

Alkanethiols with amine and carboxyl end groups are commonly used to immobilise proteins onto sensor surfaces [4, 5]. Mercaptoundecanoc acid (MUDA) is a well-known alkanethiol molecule that provides a well ordered self assembled monolayer. For this reason MUDA was chosen as a surface coating for the Biacore sensor chip. Moreover, other surface chemistries using cysteamine SAMs and dendrimers to increase the capacity of the sensor surface for molecular immobilisation were also considered.

Poly(amidoamine) (PAMAM) dendrimers are large, branching molecules formed by repeated addition of functional groups to an ethylenediamine core PAMAM dendrimers range in diameter from about 2 nm (generation 1) up to about 13 nm (generation 10) [6]. The molecular weight and number of peripheral groups of dendrimers increase exponentially with each generation, while the diameter increases more or less linearly [7]. PAMAM dendrimers are reported to have robust, covalently fixed, three-dimensional structures, which provide a

high density of terminal amino, carboxyl or hydroxyl groups at the outer surface [7]. Dendrimers possess a large number of end groups per molecule and thereby increase surface functionality [8]. The properties of dendrimers make them ideal molecular binding blocks for a wide range of applications involving self-assembled monolayers, which can be used for chemical sensing purposes [9, 10, 11, 12]. For example, a 4th generation PAMAM dendrimer having ferrocenyl units tethered to some of the surface groups could be used as a sensing layer within an electrochemical sensor by Yoon and co-workers [13] and have also been applied for protein and DNA immobilisation [14, 15, 16, 17]. Dendrimer activated solid supports have also been used for nucleic acid and protein microarrays by Benters and co-workers [18].

Dendrimers are synthetic highly ordered polymeric structures unlike other polymer based nanoparticles. The metal based nanoparticles (such as Au or Ag) allow physical adsorption of biomolecules,however a tailored surface chemistry is needed for biomolecule immobilisation or surface attachment. The ordered structure of dendrimers which can be obtained as different generations not only provides different size nanoparticles but also defined end groups, opening an easy way to either immobilise the dendrimers to a surface or attachment of the biomolecules to the dendrimer. Therefore, in this work, a 4^{th} generation dendrimer was used to investigate its effect on increasing the immobilization capacity of proteins and DNA molecules on 1 %, 10 %, and 100% (2 mM) MUDA coated surfaces and compared to other types of surface modification such as cysteamine coated surfaces. The purpose of this is to develop the most appropriate immobilization surface for both protein and DNA assays (Scheme 1).

2. MATERIAL AND METHODS

2.1. Materials and reagents

Mercaptoundecanoic acid (MUDA), mercaptoundecanol (MUDO), biotinylated-BSA, amino terminated generation 4 polyamidoamine (PAMAM) dendrimer, cysteamine, spectrophotometric grade ethanol, phosphate buffered saline (PBS, 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4), Hydrochloric acid (HCI), Tris-HCI, sodium chloride (NaCI), ethylenediaminetetraacetic acid (EDTA) and all oligonucleotides were purchased from Sigma-Aldrich (Poole, UK). 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and NeutrAvidin (NA) and EZ-link-sulfo-NHS-Biotin was purchased from Pierce-Thermo Scientific (Cramblington, UK). Oxygen free nitrogen was purchased from BOC (Manchester, UK). Ultrapure water (18 MΩ cm^{-1}) was obtained from a Milli-Q water system (Millipore Corp., Tokyo, Japan). Automated Biacore 3000 biosensor and bare gold sensor chips (GE Healthcare, Sweden) were used for the experiments. Oligonucleotides were bought from Sigma-Genosis (Poole, UK). Oligonucleotides sequences used in the study can be listed as; surface capture probe: 5' [Biotin] CAGCTTTGAGGTGAGTGTTTGTGCCTGTCC, scrambled capture probe: 5' [Biotin] CCATCGGCATGTACCGTATCGGCGCGT and target probe: 5' [Biotin] GGACAGGCACAAACA ATCACCTCAAAG. Here the target probe was selected with biotin for possible inclusion of an amplification step, however, a probe without biotin should otherwise be used in the assay.

2.2. Sensor chip cleaning and SAM deposition

Sensor chips were firstly cleaned with nitrogen plasma for one minute, and then rinsed with ethanol and kept in ethanol until used. Both a 2 mM MUDA and a 2 mM MUDO solution were prepared in ethanol. To obtain 1 %, 10%, 100 % MUDA SAMs these coating solutions

were prepared using the 2 mM MUDA and 2 mM MUDO stocks. For example, 1% MUDA was prepared through mixing 1 ml of 2 mM MUDA and 99 ml of 2 mM MUDO. In the case of 100% MUDA, 2 mM MUDA stock solution was directly used with no addition of MUDO. The selected chips were put in different concentrations of MUDA solutions overnight at room temperature under dark conditions. The MUDA coated chips were then washed with ethanol rinsed with water and dried using a nitrogen stream.

For cysteamine coating, selected clean chips were put in 5 mM cysteamine solution prepared in methanol in a Petri dish and then covered with aluminium foil and incubated overnight at ambient temperature. The cysteamine coated chips were then washed with methanol, rinsed with water and dried using a nitrogen stream. The chips were kept in the refrigerator until used.

2.3. Sensor surface modification methods

An automated Biacore 3000 biosensor and bare gold sensor chips were used for this work. The operating temperature of the assays was 25ºC and the flow rate of the buffer was 10 µl min^{-1} throughout the assays. Biacore sensor chips possess four sensing spots each, enabling the measurement of active and control sensor surfaces simultaneously.

Two different methods were considered for surface modification using the PAMAM dendrimers. Biotin modified dendrimers were formed on either 1% , 10% or 100% MUDA monolayers and the results of NeutrAvidin capture was compared with the surfaces that were coated with MUDA or biotin-modified cysteamine (Scheme 2). The fabrication methods used for the dendrimer layers are described below:

Method 1 - *Capture of NeutrAvidin on to biotinylated dendrimer immobilised 1%, 10% or 100% MUDA surface:* Initially sensor chips were coated with 10% (or 1%) MUDA and 90% (or 99%) mercaptoundecanol as described in section 2.2. Following modification the sensor chips were docked to the Biacore 3000 instrument and primed with degassed PBS that was used as running buffer. The sensor surface was first activated with a 1:1 mixture of 400 mM EDC and 100 mM NHS (final concentrations: 200 mM EDC and 50 mM NHS). EDC– NHS solution was then injected onto the sensing spot (3 min). This was followed by two injections of 5% amino dendrimer (MW: 7332) for 5 and 2 minutes, respectively. Then 1M ethanolamine was injected for 3 minutes to the sensor surface to block any remaining active sites. To modify the amino terminal groups of the dendrimer with biotin, sulfo-NHS-biotin was injected twice over the sensing spot for 5 and 3 minutes. The sensor surface was now biotinylated and ready for NeutrAvidin capture. After the sensor surface biotinylation was completed, the sensor chip was then docked to the biosensor system. After priming with PBS, a 100 μg ml^{-1} NeutrAvidin solution was injected for 3 minutes to the sensor surfaces. For some of the Biacore sensor chips amino dendrimer immobilisation and surface biotinylated was performed separately in a Petri dish.

Method 2 - *Immobilisation of NeutrAvidin on to 1%, 10% or 100% MUDA coated sensor surface*: The immobilisation was achieved using conventional amine coupling chemistry on MUDA coated sensor chips [19]. Running buffer used for the immobilisation was degassed PBS. Sensor surfaces were first activated with a 1 : 1 mixture of 400 mM EDC and 100 mM NHS, prepared in 0.22 μm filtered deionised water, and mixed immediately prior to use (final concentrations: 200 mM EDC and 50 mM NHS). EDC– NHS was injected simultaneously across the sensor surface for 3 min. A 100 μg ml^{-1} NeutrAvidin solution was injected for 3

minutes. The sensor surface was then blocked with 1 M ethanolamine, pH 8.5 for 3 minutes. The response changes were recorded 2 minutes after the protein injection was completed.

Method 3 - *Capture of NeutrAvidin on to biotinylated cysteamine ($SH\text{-}C_2H_4\text{-}NH_2$) surface*: Initially the sensor surface was modified with a 5 mM solution of cysteamine in methanol using the protocol described in Method 1. Following this 2.5 mg EZ link-Biotin-LC-NHS was dissolved in 200 µl PBS and added to the sensor chips in a Petri dish and incubated at 25ºC. The sensor chips were then washed with PBS and deionised water several times and dried with a nitrogen stream. Once the sensor surface biotinylation was complete, the sensor chip was docked to the biosensor instrument. After priming with PBS, a 100 µg ml^{-1} NeutrAvidin solution was injected for 3 minutes to the sensor surfaces.

2.4. Immobilization or capture of NeutrAvidin on the surfaces

On the biotin-modified dendrimeric surface and biotin modified-cysteamine SAM surface the NeutrAvidin was captured via affinity interactions with the biotinylated surface whereas it was immobilized on the non-dendrimer SAM surfaces obtained with MUDA through standard EDC/NHS chemistry. In the former case, the NeutrAvidin was captured by its affinity for biotin and the injection of NeutrAvidin was performed for 2 times for 3 minutes. In later case, EDC/NHS was injected to the sensor chip surface and NeutrAvidin was then directly immobilized to the surface before ethanolamine blocking. The immobilization of NeutrAvidin was applied as two 3 minutes additions of 100 µg ml^{-1} NeutrAvidin solution. In both procedures, PBS was used as running buffer during the NeutrAvidin injections.

2.5. Testing of the surfaces with protein and DNA assays

In protein assays the used target protein was biotinylated-BSA, thus after NeutrAvidin injection, to check for non-specific binding, BSA was injected onto the sensor surface before biotinylated BSA. A 5 μg ml^{-1} target protein (biotinylated-BSA) was used for the binding reaction and the recorded RU was compared to non-specific BSA binding. During the protein assay PBS was used as the running buffer.

For the DNA assay after NeutrAvidin modification of the sensor surface the running buffer was changed to Tris buffer (10 mM Tris-HCI, 1 M EDTA, NaCI, pH: 7.0). Later complementary and scrambled surface probes were captured on the NeutrAvidin immobilised sensor surfaces and hybridisation of a target probe was investigated. The sequences of the used oligonucleotides were; surface capture probe: 5' (Biotin) CAGCTTTGAGGTGATTGTTTGTGCCTGTCC, scrambled capture probe: 5' (Biotin) CCATCGGCATGTACCGTATCGGCGCGT and target probe: 5' (Biotin) GGACAGGCACAAACAATCACCTCAAAG. (The used target probe was a region of *p53* gene in which one of the most common point mutation of lung cancer occurs). After surface and scrambled probes were captured on NeutrAvidin surface (5 min injection), the remaining NeutrAvidin active sites were blocked using 10 mM biotin (1 min injection). The response changes due to target hybridisation were recorded 5 min after the injection started. Higher concentrations of DNA probes were initially utilised and 1 μM concentration of each probe was then used during the assay.

3. RESULTS AND DISCUSSION

3.1. NeutrAvidin immobilization on dendrimer modified or flat surfaces

Initially, bare gold Biacore 3000 sensor chips were modified with 1 % 10% and 100 % MUDA and cysteamine and later amino terminated generation 4 PAMAM dendrimers were immobilised onto MUDA coated Biacore sensor chips. Subsequently cysteamine coated and dendrimer immobilised sensor chips were modified with Sulfo-NHS-Biotin (methods 1 and 3, section 2.3). Following this sensor chips were docked to the instrument and the capture of NeutrAvidin was performed using 3 minutes injection of 100 μg ml^{-1} NeutrAvidin solution. The results showed that the highest NeutrAvidin capture was observed when sensor chips modified with biotinylated dendrimer that were immobilised on 1% MUDA were used. The NeutrAvidin capture response was 1.5 times higher than the response obtained from a planar biotinylated surface (Figure 1). The capture of NeutrAvidin on biotin-dendrimer layer that were immobilised on 10% MUDA gave 1.3 times higher response than NeutrAvidin captured on a flat biotin surface. These results clearly show that the use of dendrimers increases the surface area of the sensor chips to a degree so that more NeutrAvidin capture occurs compared to a planar surface. Surface area did increase with the use of 1% MUDA for the immobilisation of the dendrimer as expected, since lower amounts of amino terminal groups of the dendrimers were used for the immobilisation of the dendrimers to the MUDA surface and hence the shape of the dendrimers are not flattened [20].

In an earlier study in 2004, Mark et al. suggested that PAMAM dendrimer (generation 4) modified surfaces caused a 2.5 fold antibody immobilisation signal enhancement with respect to a flat SAM surface within a SPR assay [15]. Two recent studies by Singh et al. and Shayir et al. has shown 1.6 times and 2 times higher immobilisation levels using generation 4 PAMAM dendrimer coated surfaces, respectively [9, 17]. In our study we observed a 1.5 times higher immobilisation levels of NeutrAvidin using the dendrimer in the sensor format studied, close to the results obtained in recent studies. After testing the NeutrAvidin

immobilisation performances of the surfaces prepared, protein and DNA capture capacity of the surfaces were also investigated to determine if dendrimer surfaces based on 1% MUDA had higher protein or DNA capture capacity.

3.2. Protein and DNA capture on NeutrAvidin modified dendrimer or flat surfaces

Protein assay:

After the NeutrAvidin immobilisation, binding of 5 µg ml^{-1} biotinylated-BSA was performed to assess the activity of the captured NeutrAvidin. As it can be seen from Figure 2A, BSA alone was also injected at 5 µg ml^{-1} concentration first for non-specific binding analysis and this resulted in responses from19 to 38 RU, indicating that non-specific binding of BSA to the surfaces were limited. The 5 µg ml^{-1} Biotin-BSA binding was slightly high on NeutrAvidin captured biotin-dendrimer on 10% MUDA surface (758 ± 43 RU) (n=10) which was followed with biotin-dendrimer on 1% MUDA surface (701 ± 33) (n=10). Although the NeutrAvidin capture capacity of all three surfaces showed similar responses (within the range of standard deviation) Figure 2B, as biotin-dendrimer on 10% MUDA had lower non-specific binding properties, this surface may be preferred to immobilise biotinylated proteins. However 1% MUDA also gave good results here and can also be applied.

DNA capture and hybridization assay:

Seven NeutrAvidin modified surfaces including cysteamine coated, 1%, 10% and 100% MUDA coated dendrimeric and non-dendrimeric surfaces were utilised for the DNA capture and hybridization assays. Since the DNA assays have been less commonly investigated in the literature using biosensors for disease detection and single point mutation analysis, a range of surface chemistry immobilisation were investigated in this work in order to allow the

selection of an appropriate surface for single stranded DNA immobilisation. The concentrations of DNA surface and target detection probes utilised were 20 µg ml^{-1} and 1 µg ml^{-1} respectively during the DNA assay. The best results for DNA capture was obtained from using MUDA coated surfaces on which the standard EDC/NHS immobilisation procedure was performed, with the efficiency of this method being highest on 1% MUDA coated surface (Figure 3A) . The recorded RU changes were 240 ± 10.6, 270 ± 7.0 and 559 ± 34.6 for DNA capture assay on 100%, 10% and 1% MUDA coated surfaces, respectively (n=10 for each). Although cysteamine coated surface and dendrimer-immobilized MUDA surfaces were promising, the obtained output was much lower than the standard EDC/NHS chemistry applied assays. The cysteamine surface gave a marginally higher response than the dendrimeric surfaces; moreover the recorded RU change in 10% MUDA-modified dendrimer surface was better than the other dendrimer surfaces. The measured responses of DNA capture assay were 89.3 ± 4.0, 69 ± 7.0, 54 ± 4.0 and 39 ± 4.0 RU for cysteamine and 10%, 100%, 1% MUDA coated dendrimer surfaces respectively (Figure 3A-DNA capture) (n=10 for each). To understand the efficiency and specificity of DNA capture on the developed surfaces, DNA hybridization assays were also performed, with two different DNA probes being used in this part of the study. The target and scrambled capture probes were injected onto separate parts of the NeutrAvidin immobilized or captured chip surface via the flow channels of the sensor. The target probe was complementary to the detection probe while the scrambled was not, to utilise the specificity of the hybridization interaction. The recorded RU changes for the hybridization reactions were found to be parallel to the DNA capture results, which indicates that higher DNA capture leads to more hybridization. The binding results of the detection probe were 320 ± 26, 253 ± 25 and 226 ± 11 RU on surfaces modified through the standard EDC/NHS method on 1%, 10% and 100% MUDA coated surfaces respectively (n=10 for each). However, the obtained RU changes for the other surfaces were between 69

and 32 RU for hybridization (Figure 3B-hybridization) and non-specific binding of the detection probe to the scrambled probe was measured as zero for all utilised surfaces as seen in Figure 3B. Figure 4, show the reproducibility of the sensor surface for the DNA hybridization reaction. As seen in the sensorgram, consistent and clear response can be obtained for each hybridization reactions after each regeneration.

Using Equation 1, the theoretical maximum DNA capture response on the NeutrAvidin surface or hybridization of target to captured probe can be calculated and hence the % activity (R_{max} = calculated response; $MW_{analyte/ligand}$ = molecular weight of analyte or ligand [DNA target MW: 8674, DNA probe MW: 9635, NeutrAvidin MW : 60 000]; R_{ligand} = response obtained from the ligand; V_{ligand} = valency of the ligand, proposed stoichiometry of the interaction).

$$R_{\max} = \frac{MW_{analyte}}{MW_{ligand}} \times R_{ligand} \times V_{ligand} \qquad \text{Eq. 1}$$

% activity results obtained from the assays performed using different surface chemistries are shown in Table 1. The observations from this study have shown that although the detection of single-stranded DNA probe and hybridization were achieved specifically on dendrimer-modified surfaces, the SPR signals and % activity of DNA capture results were lower when compared to the surfaces modified with MUDA and modified using EDS-NHS chemistry.

Although dendrimeric surfaces resulted in higher levels of NeutrAvidin immobilisation with respect to non-dendrimeric surfaces, lower DNA capture and hence hybridisation responses suggest that the biotin binding sites of the immobilised NeutrAvidin was limited possibly due to steric hindrance due to the structure of the dendrimer. When 1 % MUDA was used as

sensor surface, the resultant widely spaced NeutrAvidin molecules allowed capture of DNA probe more efficiently.

Mark et al [15] studied dendrimer-functionalised SAM surfaces for both protein and DNA immobilization through an SPR-based sensor and they showed that when using dendrimer immobilized surfaces, DNA immobilization was achieved with high specificity and the same chip surface could be regenerated without activity loss for further hybridization reactions. However, they only tested two surfaces, one included only a gold-thiol SAM whilst the other utilised dendrimer molecules. While in our work we used two different SAM coatings (MUDA and cysteamine) to develop various surfaces with or without dendrimer molecules. Similar to Mark et al.'s work, in our study we also observed an increased NeutrAvidin immobilisation capacity for the dendrimer-modified surface on MUDA SAM.

In similar work, Nelson et al [21] designed mixed SAMs for streptavidin immobilisation and surface plasmon resonance (SPR) was employed to characterize the films produced on gold from a range of binary mixtures of a biotinylated alkylthiol (BAT) and either a C16 methyl-terminated thiol (mercaptohexadecane, MHD) or a C11-oligo(ethylene glycol)-terminated (OEG) thiol in ethanol. The obtained composite indicated the relationship between the specificity of streptavidin recognition and the surface architecture and properties of the mixed SAMs. Pure BAT did not assemble into ordered monolayers, but addition of the MHD thiol to the solutions of BAT improved the orientation of the biotinylated thiol and increased the order within the hydrocarbon region of the film. The addition of the OEG thiol to solutions of BAT also improved the orientation of the biotinylated thiolas well as promoting specific adsorption of streptavidin by increasing the concentration of exposed biotin groups above the film.

Various generations of PAMAM dendrimer were also employed for the development of DNA chips by different research groups [22, 23]. Lim and colleagues [24] studied 3rd generation PAMAM dendrimer-modified surfaces using interaction between avidin and biotin molecules to improve DNA chip properties and showed that dendrimer coating increased the fluorescence intensity due to hybridisation of target DNA tagged with fluorescence dye. Mannelli et al. [25] developed DNA immobilisation procedures in an SPR-based biosensor modified using 11-mercaptoundeconoic acid-poly(ethylenimine) and dextran coatings, forming two different multilayers on the surface that were linked by covalent bonds. They studied, several mutations related to human cystic fibrosis with a real-time assay approach, measuring interactions between immobilised probes and hybridization probes. These were monitored using a flow rate of 50 µl min^{-1} over 15-30 minutes and although the method was more complex, better results were found when dendrimer was used.

In this study we investigated the impact of PAMAM dendrimers on increasing the binding capacity of protein and DNA molecules on SAM coated sensor chip surfaces using SPR as the sensing method. Dendrimer modified surfaces increased the immobilisation capacity of NeutrAvidin when compared to planar surfaces; however, this effect was not observed for further protein capture on NeutrAvidin layer using the SPR sensor chip. Planar MUDA surfaces gave the best results for DNA capture and hybridisation assays, and dendrimer modified surfaces did not lead to any increase for DNA binding when compared to the MUDA surfaces.

4. CONCLUSION

In this study, two types of alkanethiols, MUDA and cysteamine, were used to construct self assembled monolayers on the bare gold SPR sensor chip surfaces for protein and DNA immobilization assays. Some of the sensor chips were further modified with PAMAM dendrimer molecules to increase the capacity of surface for both protein and DNA immobilisation. Compared to MUDA or biotin-modified cysteamine surfaces, higher NeutrAvidin immobilisation responses were obtained from the dendrimer-modified surfaces, in particular, NeutrAvidin immobilisation capacity was 50% higher for the dendrimer surface prepared on 1% MUDA. However, higher NeutrAvidin immobilisation capacity of the dendrimer surfaces was not translated into further protein or DNA capture on NeutrAvidin. While protein (biotinylated-BSA) capture responses on NeutrAvidin immobilised dendrimer layers were not very different from the MUDA or biotin modified cysteamine surfaces, DNA immobilisation responses on NeutrAvidin modified dendrimer surfaces were lower with respect to NeutrAvidin immobilised MUDA or biotin modified cysteamine.

In summary, the observed results demonstrate that thedendrimer modified 1% MUDA surface is the most convenient surface for protein assays whereas 1% MUDA coating leads to the best output for DNA assays after which standard EDC/NHS method are utilised to immobilise NeutrAvidin for DNA capture.

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Table 1: % activity responses for DNA probe capture and target hybridisation were calculated for the assays on different surface chemistries.

Scheme 1: The different surface chemistry modifications developed on the gold sensor chips.

Scheme 2: Principle of the applied protein and DNA assays on the dendrimer modified MUDA-SAM.

Figure 1: Overall comparison of the developed surfaces for NeutrAvidin capture.

Figure 2: A) Sensorgram of the protein assay on NeutrAvidin captured surfaces; biotin-cysteamine, biotin-dendrimer on 1% MUDA and biotin-dendrimer on 10% MUDA surfaces. B**)** Binding of BSA and biotinylated-BSA on NeutrAvidin immobilised on different biotinylated surfaces. A 5 µg ml^{-1} BSA and 5 µg ml^{-1} biotin-BSA were used.

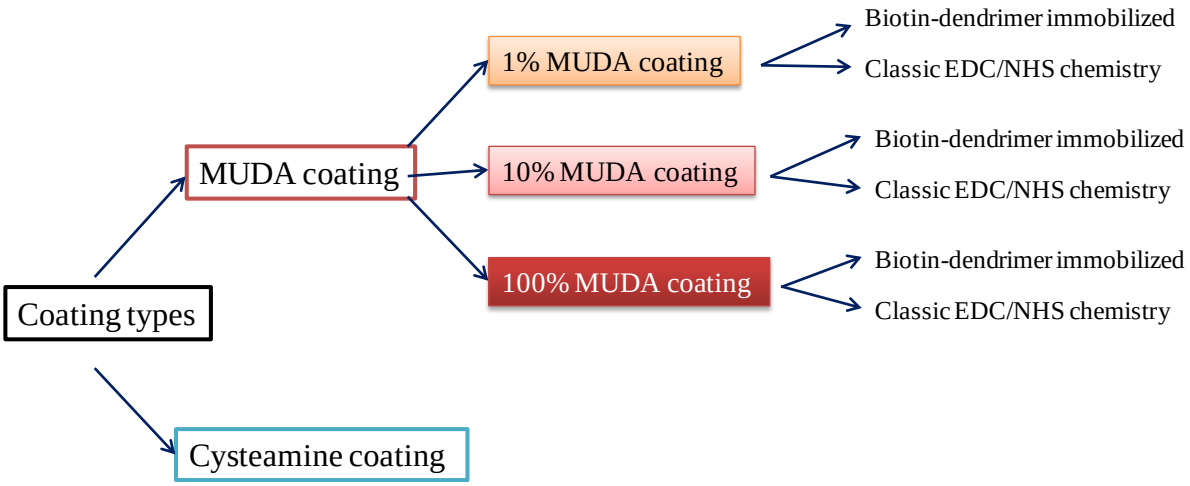
Figure 3: DNA capture and hybridization on differently modified surfaces **(a)**. Sensorgram of the DNA hybridization assay **(b)**. The lowest line shows the binding of the scrambled probe as a negative control for each surface. From bottom to top the hybridization on 1% MUDA-modified dendrimeric surface, 100% MUDA-modified dendrimeric surface, cysteamine surface, 10% MUDA-modified dendrimeric surface, 100% MUDA, 10% MUDA and 1% MUDA surfaces.

Figure 4: Reproducibility of the sensor surface for DNA hybridization reaction.

Table 1: % activity responses for DNA probe capture and target hybridisation were calculated for the assays on different surface chemistries.

Sensor Surface	**Probe capture % activity**	**Hybridisation % activity**
1% MUDA + dendrimer	3	91
100% MUDA + dendrimer	5	99
10% MUDA + dendrimer	6	97
biotin-cysteamine	9	69
100% MUDA + EDC/NHS chemistry	32	105
10% MUDA + EDC/NHS chemistry	30	104
1% MUDA + EDC/NHS chemistry	58	64

Scheme1


Coating types
MUDA coating
1% MUDA coating
10% MUDA coating
100% MUDA coating
Biotin-dendrimer immobilized
Classic EDC/NHS chemistry
Biotin-dendrimer immobilized
Classic EDC/NHS chemistry
Biotin-dendrimer immobilized
Classic EDC/NHS chemistry
Cysteamine coating

Scheme 2

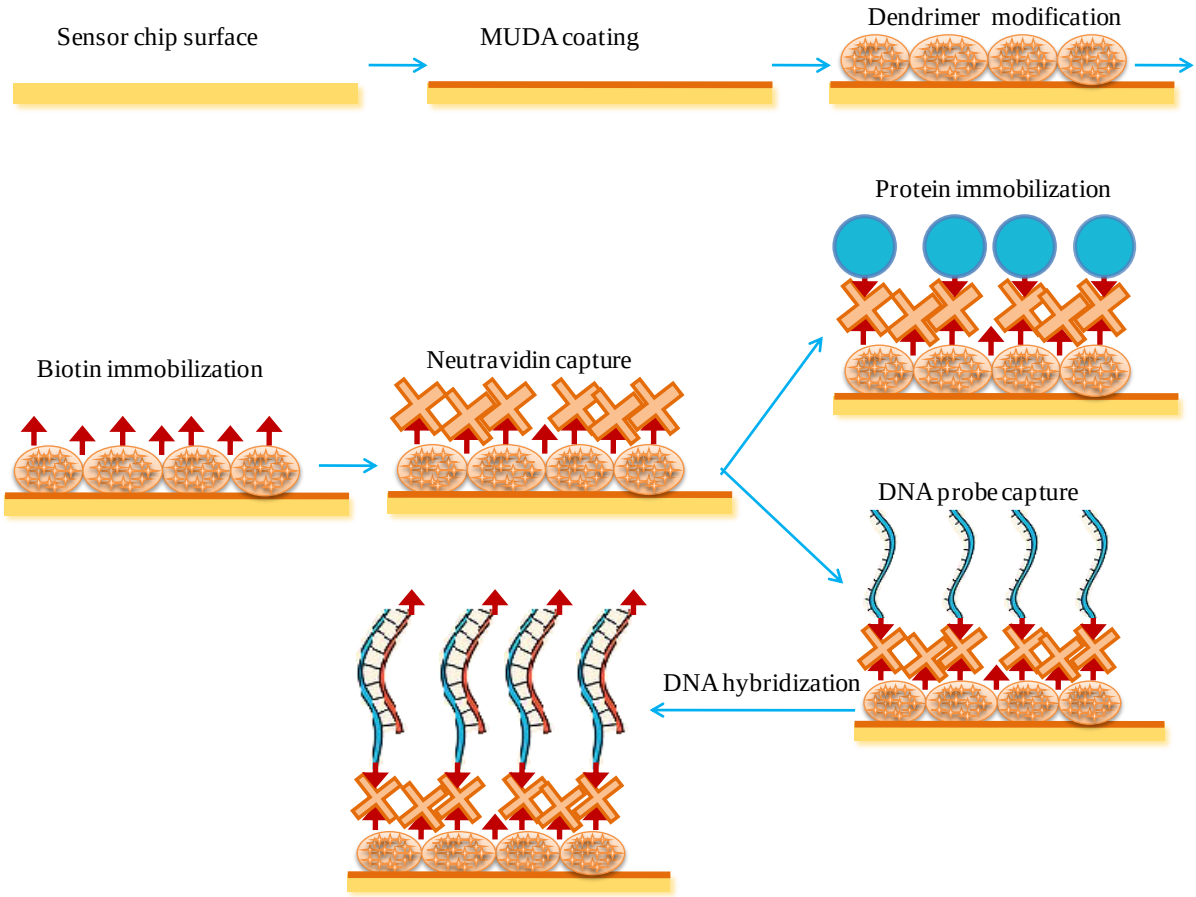

Sensor chip surface
MUDA coating
Dendrimer modification
Protein immobilization
Biotin immobilization
Neutravidin capture
DNA probe capture
DNA hybridization

Figure 1

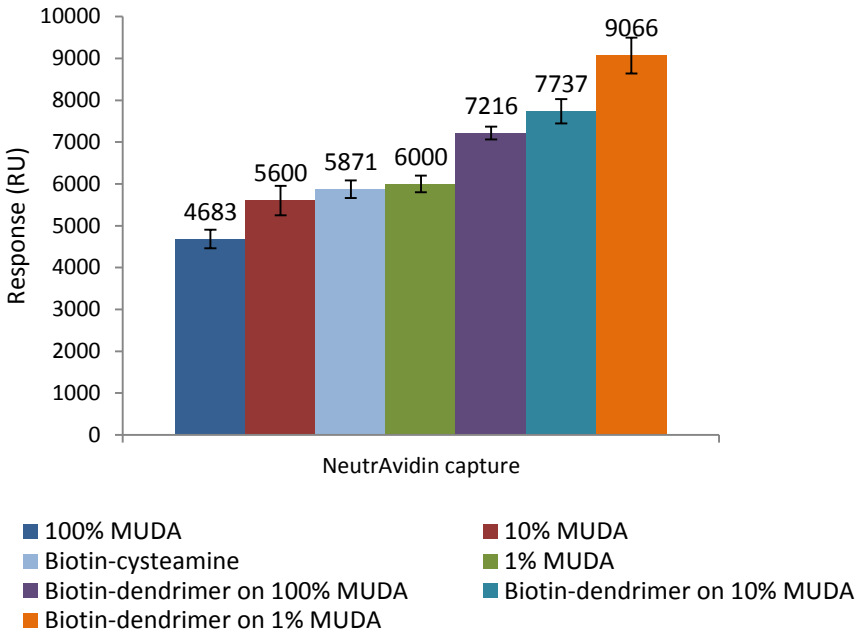
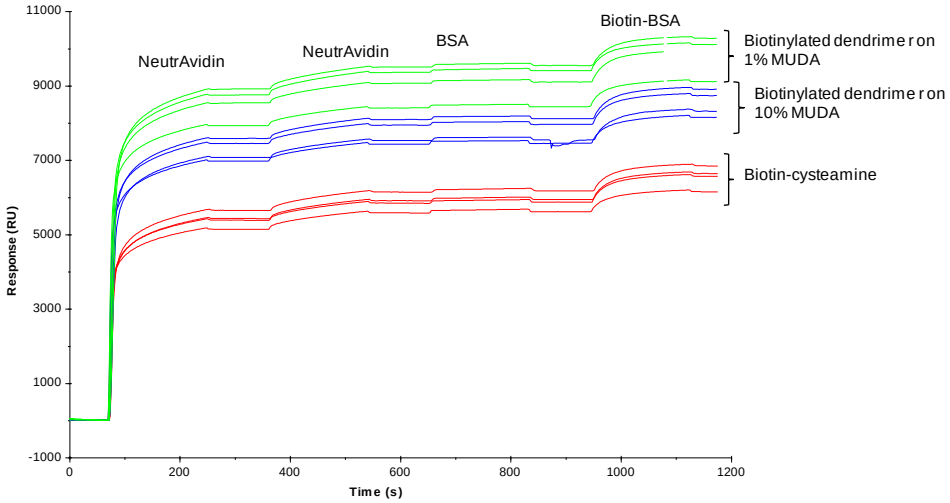

10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Response (RU)
4683
5600
5871
6000
7216
7737
9066
NeutrAvidin capture
100% MUDA
10% MUDA
Biotin-cysteamine
1% MUDA
Biotin-dendrimer on 100% MUDA
Biotin-dendrimer on 10% MUDA
Biotin-dendrimer on 1% MUDA

Figure 2

A)


11000
9000
7000
5000
3000
1000
-1000
Response (RU)
0
200
400
600
800
1000
1200
Time (s)
NeutrAvidin
NeutrAvidin
BSA
Biotin-BSA
Biotinylated dendrimer on 1% MUDA
Biotinylated dendrimer on 10% MUDA
Biotin-cysteamine

B)

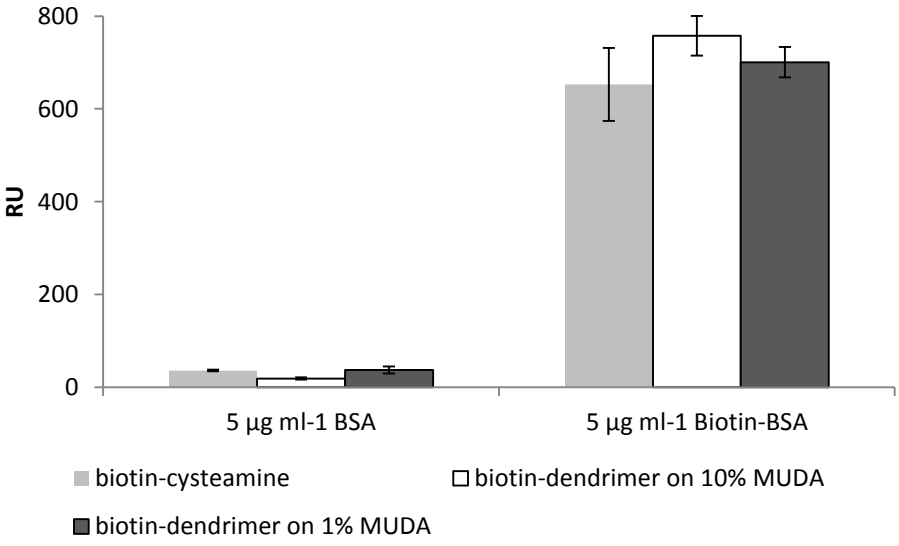
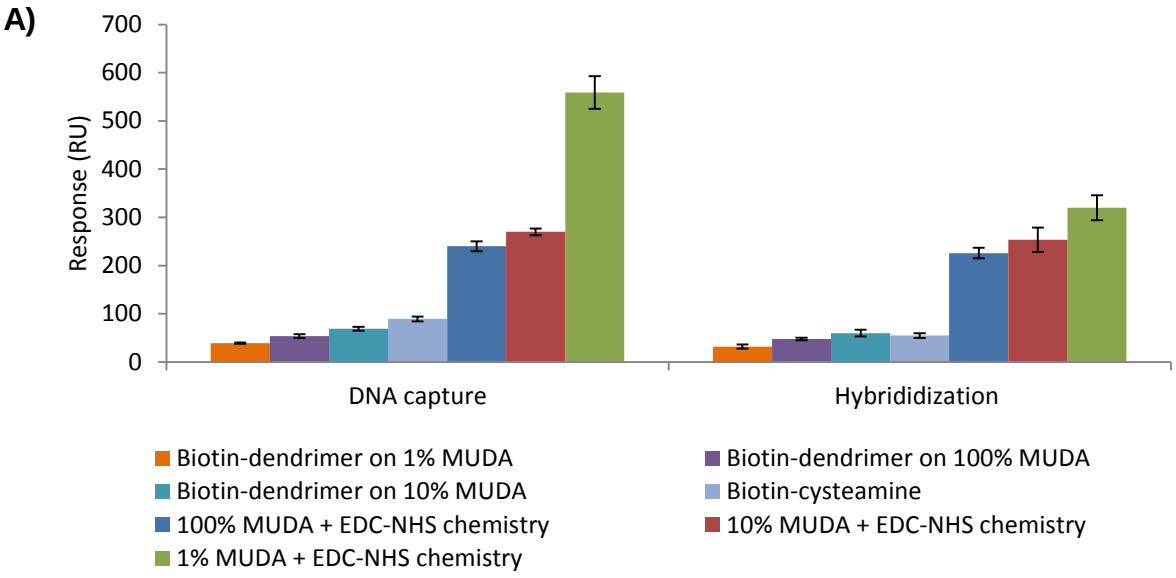

800
600
400
200
0
RU
5 µg ml-1 BSA
5 µg ml-1 Biotin-BSA
biotin-cysteamine
biotin-dendrimer on 10% MUDA
biotin-dendrimer on 1% MUDA

Figure 3


A)
Response (RU)
700
600
500
400
300
200
100
0
DNA capture
Hybridididization
Biotin-dendrimer on 1% MUDA
Biotin-dendrimer on 100% MUDA
Biotin-dendrimer on 10% MUDA
Biotin-cysteamine
100% MUDA + EDC-NHS chemistry
10% MUDA + EDC-NHS chemistry
1% MUDA + EDC-NHS chemistry

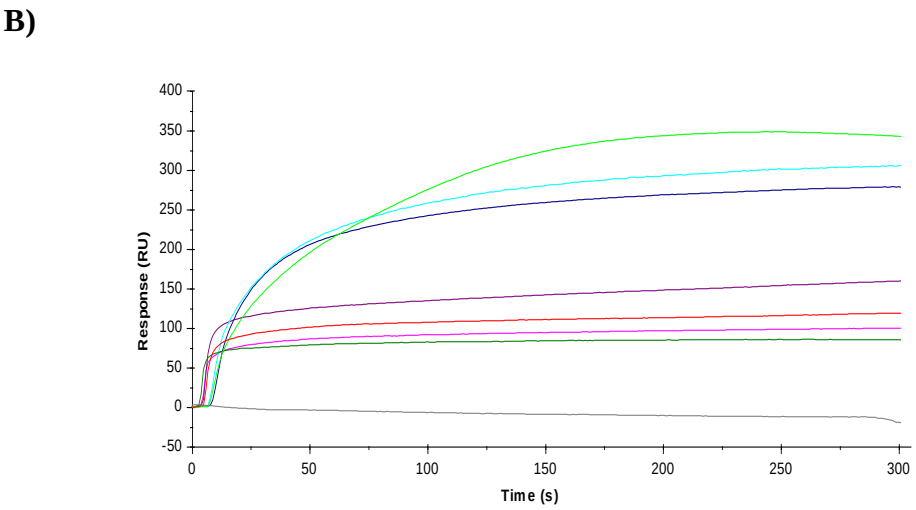
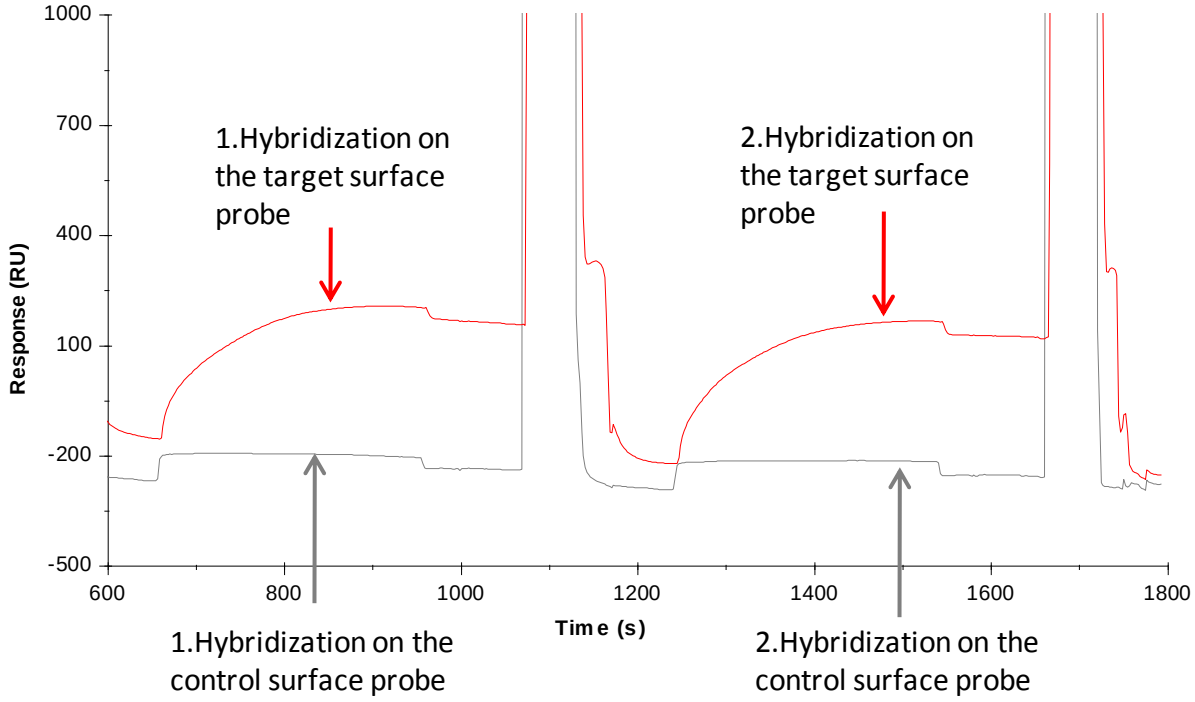

B)
Response (RU)
400
350
300
250
200
150
100
50
0
-50
0
50
100
150
200
250
300
Time (s)

Figure 4


1000
700
400
100
-200
-500
Response (RU)
600
800
1000
1200
1400
1600
1800
Time (s)
1.Hybridization on the target surface probe
2.Hybridization on the target surface probe
1.Hybridization on the control surface probe
2.Hybridization on the control surface probe