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IMPROVED PROCEDURES FOR IMMOBILISATION OF OLIGONUCLEOTIDES ON GOLD-COATED PIEZOELECTRIC QUARTZ CRYSTALS

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Abstract

The high sensitivity and specificity of DNA hybridisation techniques makes them powerful tools for environmental or clinical analysis. This work describes the development of a DNA piezoelectric biosensor for the detection of the hybridisation reaction. Attention was focused on the choice of the coating chemistry that could be used for the immobilisation of oligonucleotides onto the gold surface of the quartz crystal. Four immobilisation procedures were tested and compared considering the amount of immobilised probe, the extent of the hybridisation reaction, the possibility of regeneration and the absence of non-specific adsorption. All the experiments were performed with oligonucleotides of 25 bases (probe, target and non-complementary oligonucleotide).The four coating methods were all based on the use of self-assembled monolayers. Three of them employed the interaction between streptavidin and biotin for the immobilisation of a biotinylated probe.Results indicated that immobilisation of a biotinylated probe on streptavidin linked to a layer of carboxylated dextran provides higher sensitivity for the detection of the hybridisation reaction, absence of non-specific adsorption and a higher stability with respect to the regeneration step.

1. Introduction

Interest in the application of piezoelectric devices for biochemical analysis has increased markedly following the elucidation of the theory of their operation in liquids and the consequent appreciation that they offer an effective alternative to optical techniques such as surface plasmon resonance and interferometry. Methods to improve the selectivity and sensitivity of piezoelectric biosensors have been pursued to facilitate their application in antigen-antibody or DNA hybridisation detection. The sensitivity can, in some cases, be improved by using crystals of higher frequencies (> 10 MHz), but these devices are often difficult to operate in liquids because of fragility and frequency stability problems (Caruso *et al.*, 1997). Alternatively, the selectivity can be improved by improving the quality of the surface of the crystal and the immobilisation procedure. Actually, the principal limitation of the quartz crystal microbalance (QCM) is non-specific adsorption of molecules present in real matrices; QCM is a mass sensor and any molecule able to bind to the surface is a potential interferent. Experiments with piezoelectric biosensors applied to antigen/antibody reactions or to DNA hybridisation clearly demonstrated that using appropriate immobilisation chemistry, non-specific binding can be effectively minimised (Tombelli and Mascini, 2000a; Zhou *et al.*, 2001).

Advances in genomics and proteomics have catalysed escalating interest in nucleic acids as bioreceptors for biosensors and biochips (Vo-Dinh and Cullum, 2000). The biorecognition mechanism involves hybridisation of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), with the complementarity of adenine:thymine (A:T) and cytosine:guanine (C:G) pairing forming the basis for the exquisite specificity in such biosensors. These analytical systems are often founded on the immobilisation of a fragment of DNA with a specific sequence (probe) followed by monitoring and recording the variation of the transducer signal when the complementary fragment (target) in solution hybridises with the probe.

In the present work, the optimisation of the coating of the gold quartz crystal surface in order to improve the immobilisation of oligonucleotide probes is reported. Four immobilisation procedures are compared:

- aminoethanethiol/probe;
- 11 mercaptoundecanoic acid/streptavidin/biotinylated probe;

- mixed thiols/streptavidin/biotinylated probe;
- 11 mercaptundecanol/dextran/streptavidin/biotinylated probe.

All the procedures involved the use of self-assembled monolayers (SAM). Functionalised self-assembled monolayers offer promising possibilities for the immobilisation of biomolecules on different solid surfaces (Allara, 1995; Gopel and Heiduschka, 1995; Mandler and Turyan, 1995; Wink *et al.*, 1997). Three of the four immobilisation procedures, involved the use of the biotin-streptavidin interaction, which has been widely used for the immobilisation of biotinylated DNA probes (Caruso *et al.*, 1997; Okahata *et al.*, 1998; Niikura *et al.* 1998, Tombelli *et al*., 2000b,c). In this investigation of the interaction of streptavidin with biotinylated oligonucleotide probes, three different coatings of the gold surface were used for the immobilisation of streptavidin: 11-mercaptoundecanoic acid, mixed thiols and thiols/dextran.

The four immobilisation procedures were compared and evaluated for the detection of the target oligonucleotides, the possibility of regeneration and the amount of non-specific adsorption. With each procedure, after the probe immobilisation, the hybridisation reaction was evaluated with the complementary oligonucleotide. Experiments were also performed with a non-complementary oligonucleotide to verify the absence of non-specific adsorption. Regeneration of the single-stranded probe was obtained using HCl 1 mM for 1 minute after each cycle of hybridisation.

2. Materials and apparatus

2.1. Materials

11-Mercaptoundecanol was synthesised in the laboratory (Miller *et al.*, 1991). Dextran was purchased from Pharmacia Biotech (Uppsala, Sweden), epichlorohydrin and N-hydroxysuccinimide (NHS) from Fluka (Milan, Italy). Ethanol and all the reagents for the buffers were purchased from Merck (Darmstadt, Germany). All other reagents were purchased from Sigma Aldrich (Milan, Italy).

Oligonucleotides were purchased from Pharmacia Biotech (Uppsala, Sweden). The sequences of bases of the oligonucleotides used in these experiments were as follows:

Probe:	5’ TGGAGGACGTGTGCGGCCGCCTG 3’
Complementary oligonucleotide:	5’ CAGGCGGCCGCACACGTCCTCCA 3’
Non-complementary oligonucleotide:	5’ AAAGTCCTGGCGCAGGACAACAC 3’

The sequence of the oligonucleotide probe is included in a region of a human gene encoding for the production of the protein Apolipoprotein E, where a point mutation occurs. This sequence was chosen with a view to application of the optimised biosensor to the determination in real samples of DNA of the polymorphism generated by this point mutation.

The composition of the buffers used for the experiments was as follows:
Immobilisation buffer: NaCl 300 mM, Na_2HPO_4 20 mM, EDTA 0.1 mM, pH 7.4.
Hybridisation buffer: NaCl 150 mM, Na_2HPO_4 20 mM, EDTA 0.1 mM, pH 7.4.

2.2. Apparatus

10 MHz AT cut quartz crystals (14 mm, 165 μm) with gold evaporated (42.6 mm^2 area, Ø 7.4 mm) on both sides were obtained by Mistral, Latina, Italy. The crystal was housed inside a methacrylate cell with only one side of the crystal in contact with the solution added to the cell well. Before coating the gold surface with the different methods, the crystal was washed with 1:1:5 solution of H_2O_2, NH_3 and distilled water, respectively.

The frequency variations were continuously recorded using a quartz crystal analyser (Model QCA917, Seiko EG&G, Chiba, Japan): the data (the resonance frequency) are displayed on the main display screen in real time and can be read directly by a computer connected to the QCA917 interface. The resonant frequency can be measured with an accuracy of 0.1 Hz and at a sample period of 0.1, 1 or 10 sec.

3. Procedures

3.1. Measurement set-up

Figure 1 shows a typical plot of the frequency *vs* time during an affinity reaction between the probe immobilised on the crystal and the target molecule in solution. Initially a stable baseline was recorded when the crystal together with the immobilised probe was in contact with buffer. In this situation the quantity of target is null both in solution and on the crystal. The buffer was then replaced with an equal volume of a solution containing the target molecule prepared using the same buffer. In the case of standard solutions only the target molecule have been present in the solution, whereas with real samples other non-specific molecules could be contained in the matrices.

The substitution of the buffer with the target solution caused a frequency decrease. At the end of the time necessary for the reaction to occur, when the frequency had reached a stable value, the surface of the crystal was washed with buffer. This step is necessary to eliminate all the molecules that could non-specifically adsorb on the crystal surface. At this point, the frequency value was recorded. The analytical data are represented by the frequency differences between this final value and the one recorded as baseline before the affinity reaction ($\Delta F=F_B-F_A$).

3.2. Aminoethanethiol

A coating procedure, based on the use of aminoethanethiol, was used for the immobilisation of the oligonucleotide probe. After the probe immobilisation, the hybridisation reaction was evaluated with the complementary oligonucleotide. Experiments were also performed with the non-complementary oligonucleotide to verify the absence of non-specific adsorption.

After cleaning, the quartz was soaked in a 5 mM aminoethanethiol hydrochloride/ethanol solution for 10 h at room temperature. The surface of the quartz was washed with distilled water and ethanol and air-dried. These treatments resulted in a self-assembled monolayer with primary amino groups being formed on the gold surface of the quartz crystal. The crystal was then immersed in N-methylimidazole buffer (0.1 M, pH 6.0) containing 0.1 M 1-ethyl-3-(3-dimethylaminopropril)carbodiimide (EDAC) and 0.05 mg/ml of the oligonucleotide probe. The

electrode was incubated for 3 h at 25°C. These conditions allow immobilisation through the 5'-phosphate groups of the probe by the formation of a phosphoramidate bond with the amine groups on the crystal. The modified crystal was washed three times with a solution of sodium dodecylsulphate (SDS) 0.25% with NaOH 0.4 M for 5 minutes to remove adsorbed oligonucleotides. The hybridisation reaction was performed at room temperature (25°C) and using hybridisation buffer for the solutions of the oligonucleotides.

3.3. 11-Mercaptoundecanoic acid

The crystal was coated with an ethanolic 1 mM solution of 11-mercaptoundecanoic acid. The crystal was left in contact with the solution for 48 h and then sonicated in ethanol and rinsed with pure water. After washing the crystal was seated in the cell. The surface of the crystal was then activated prior to covalent coupling with 200 μl of a solution of NHS (50 mM) and EDAC (200 mM) in water. After 5 minutes the activating solution was replaced by a streptavidin solution at 200 μg/ml in acetate buffer of 10 mM, pH 5. After 20 minutes, the residual reacting sites were blocked with 200 μl of a solution of ethanolamine hydrochloride (pH 8.6, 1 M water solution). Following washing with the immobilisation buffer, the biotinylated probe was added (200 μl of a solution containing 8.5 μg/ml of probe in immobilisation buffer). The immobilisation was allowed to proceed for 20 minutes. The hybridisation experiments were then performed as reported in the previous sections.

3.4. Mixed thiols

After the cleaning treatment, the gold surface of the quartz crystal was coated with a solution 0.5 mM of 11-mercaptoundecanic acid and 0.5 mM of 1-octanethiol. The crystal was left in contact with the solution, in the dark for 24 h. The crystal was then rinsed with ethanol and water and sonicated in ethanol for 10 minutes. After washing with milliQ water the crystal was seated in the cell. The carboxylic groups of the thiol were used to immobilise streptavidin (200 μg/ml) and then the biotinylated probe, as reported in the previous section.

3.5. Thiols and dextran

The gold on the quartz was modified with 11-mercaptoundecanol and carboxylated dextran to immobilise streptavidin and then the biotinylated oligonucleotide. This immobilisation procedure was selected from a previous work where several procedures were compared (Storri *et al.*, 1998a, b). The freshly cleaned crystal was immersed in an unstirred 1 mM ethanolic solution of 11-mercaptoundecanol at room temperature, in the dark, for 48 h. The crystal was then washed with ethanol and milliQ water and sonicated for 10 minutes in ethanol to remove the excess of thiol. The hydroxylic surface was then treated with a 600 mM solution of epichlorohydrin in a 1:1 mixture of 400 mM NaOH and bis-2-methoxyethyl ether (diglyme) for 4 h. After washing with water and ethanol, the crystal was immersed for 20 h in a basic dextran solution (3 g of dextran in 10 ml of NaOH 100 mM). The surface was further functionalised with a carboxymethyl group using bromoacetic acid (1 M solution in 2 M NaOH for 16 h). All the reactions were performed at room temperature. The crystal was then washed with milliQ water and placed in the cell. The surface of the crystal was then activated prior to covalent coupling with 200 μl of a solution of NHS 50 mM and EDAC 200 mM in water. After 5 minutes the activating solution was replaced by a streptavidin solution 200 μg/ml in acetate buffer 10 mM, pH 5. After 20 minutes, the residual reacting sites were blocked with 200 μl of a solution of ethanolamine hydrochloride (pH 8.6, 1 M water solution). After washing with the immobilisation buffer, the biotinylated probe was added (200 μl of a solution 1.0 μM of the probe in immobilisation buffer). The immobilisation was allowed to proceed for 20 minutes. At this final stage the crystal was ready for the first cycle of hybridisation: the hybridisation was performed using the hybridisation buffer for all the solutions.

4. Results and Discussion

4.1. Aminoethanethiol/probe

An ideal monolayer is depicted as perfectly aligned, closely packed alkane chains, attached to a smooth surface. Alkylthiols from dilute solution form a densely packed monolayer in less than 1 h. The adsorption time seems to be independent of the chain length, but high concentrations lead to shorter adsorption times. Although dense monolayers assemble quickly, well-ordered monolayers can take days to form (Kim *et al.*, 1993). An uncontaminated gold surface is important even if the

high affinity of the thiol moiety for gold displaces contaminants (Troughton *et al.*, 1988; Keller *et al.*, 1992). Most commonly, possible contaminants are removed using cleaning solutions (often called “piranha”) to produce a hydrophilic gold surface.

The first procedure reported for the modification of the gold surface of a quartz crystal with alkylthiols and biomolecules is based on the adsorption of a functionalised alkythiol layer on the gold electrode, activation of this layer by a coupling reagent and subsequent covalent immobilisation of the receptor molecules. The immobilisation of single-stranded DNA (ssDNA) on a gold electrode with a self-assembled aminoethanethiol monolayer has been reported by Sun *et al.* (1998). In the presence of water-soluble carbodiimide reagent, the 5’-terminal phosphate end of ssDNA formed a phospharamidate bond with the primary amino group of the aminoethanethiol monolayer on the electrode surface. The molecule of aminoethanethiol can be strongly adsorbed on a gold surface through the thiol group, forming a very stable self-assembled monolayer (Chidsey *et al.*, 1990).

In the current work, this coating procedure proved suitable for the immobilisation of the oligonucleotide probe resulting in a frequency shift of 61 Hz (± 18 Hz). The interaction of the probe with the non-complementary oligonucleotide did not result in a measurable frequency shift, demonstrating the absence of non-specific adsorption on the crystal surface. The hybridisation reaction with the complementary oligonucleotide (0.75 and 1.0 μM) produced a measurable and reproducible frequency shift, but it was not possible to measure lower concentrations of the oligonucleotide (Curve 1 in Figure 2). It was possible to perform 5/6 cycles of hybridisation-regeneration, using HCl 1 mM as regenerating solution, on the same side of the crystal, i.e. with the same probe. The regeneration time was decreased from 1 minute to 30 seconds in an attempt to reduce the effects of this step on the immobilised probe and the aminoethanethiol layer. The contact with the acid solution probably damaged the binding between the probe and the thiol layer.

4.2. 11-Mercaptoundecanoic acid/streptavidin/biotinylated probe

The biotin-streptavidin interaction has some unique characteristics that make it ideal as a general bridge system in many applications (Diamandis and Christopoulos, 1991; Savage *et al.*, 1992). The non-covalent interaction of streptavidin with biotin is characterised by an affinity

constant of 10^{15} M^{-1}. This high affinity ensures that, once formed, the complex is not disturbed by change in pH, the presence of chaotropes or manipulations such as multiple washing when the complex is immobilised.

From the results obtained from the immobilisation of streptavidin ($\Delta F = 353 \pm 27$ Hz) and the biotinylated probe ($\Delta F = 55 \pm 9$ Hz), we can conclude that the immobilisation procedure involving the interaction between streptavidin and biotin is suitable for the immobilisation of an oligonucleotide probe to a gold surface of a quartz crystal. The frequency shifts induced by the hybridisation reaction were detectable with all the tested concentrations in the range 0.12-1.0 μM with a CV_{av} of 20% (Curve 2 in Figure 2). The interaction of the immobilised probe with a non-complementary oligonucleotide resulted in a measurable frequency shift of 16 Hz (± 4 Hz), which indicates the presence of non-specific adsorption. The regeneration by a 1' treatment with HCl 1 mM was possible for about 7 or 8 cycles without loss of sensitivity.

4.3. Mixed thiols/streptavidin/biotinylated probe

Mixed monolayers, consisting of substituted and non-substituted alkanethiols, offer the possibility to "dilute" substituted alkanethiols in order to have anchor groups available for immobilisation procedures in which steric hindrance is possibly reduced (Wink *et al.*, 1997). The mole ratio of a mixture of thiols in solution results in the same ratio in the mixed SAM. The two components do not phase segregate into islands. This interesting feature can be exploited to immobilise biomolecules in such a manner that steric hindrance between these molecules and their binding partners is avoided. The co-adsorption process in itself will not introduce any significant amount of effects or disorder domains within the monolayer.

This immobilisation procedure resulted in a good immobilisation of streptavidin ($\Delta F = 254 \pm 33$ Hz). A measurable frequency shift of 36 Hz (± 13 Hz) was obtained from the immobilisation of the biotinylated probe. The hybridisation of the probe with the complementary oligonucleotide produced measurable frequency shifts with all the three concentrations (0.25, 0.50 and 1.0 μM), but the results were not very reproducible having a coefficient of variation (CV) of 50% in all three cases. The frequency shift increased increasing the concentration of the oligonucleotide (Curve 3 in Figure 2). The interaction of the probe with the non-complementary oligonucleotide resulted in a

measurable frequency shift of 24 Hz (± 14 Hz). This demonstrates the presence of non-specific adsorption of the oligonucleotide on the streptavidin layer or on the gold surface. This non-specific adsorption was absent in the coating procedure with aminoethanethiol or 11-mercaptoundecanoic acid alone indicating that this monolayers of thiols could be better ordered and cover the gold surface in a more efficient way than in the case of mixed monolayers. It was only possible to perform 2 cycles of hybridisation-regeneration on the same side of the crystal. Probably, the interaction with 1 mM HCl either damaged the immobilisation of streptavidin on the monolayer or the monolayer itself.

4.4. Thiols and dextran

Dextrans are hydrophilic and non-charged natural polymeric carbohydrates, which are soluble in water in any proportion and form highly hydrated hydrogels. Due to these properties, dextrans show very low non-specific interactions with proteins. Owing to the high concentration of hydroxyl groups in the dextran molecule, chemical modification of these polymers is possible without significantly affecting their hydrophilicity. The essentially non-branched polymer chains are highly flexible and ligands immobilised in dextran matrices are well accessible (Piehler *et al.*, 1999). Hence, dextran-based polymers have found application in several fields of biochemistry and biotechnology (Lofas and Johnsson, 1990; Lofas *et al.*, 1993; Piehler *et al.*, 1996). Another feature is the low non-specific binding to the matrix of components in various samples under conditions of high ionic strength, which can be attributed to a combination of the high hydrophilicity of the surface and entropic effects due to the flexible polymer chains.

The immobilisation of streptavidin on carboxylated dextran resulted in a measurable frequency shift of 300 Hz with a CV of 20%. This is an acceptable reproducibility considering the several steps that are necessary for the coating of the gold surface. The binding of the biotinylated probe to the layer of streptavidin gave an average frequency shift of 103 Hz (± 37 Hz) which was higher in comparison to the previous immobilisation methods. Curve 4 in Figure 2 shows the average frequency shifts relative to the hybridisation of the immobilised probe increasing the concentration (0.1-1.0 μM) of complementary oligonucleotide. These measurements were performed on different crystals or on the same crystal using the regeneration procedure with HCl 1 mM. Non-specific binding was also investigated: measurements with the non-complementary

oligonucleotide were performed with solutions 0.50 and 1.0 μM. It is notable that the measurements obtained with the non-complementary strand did not result in a measurable frequency shift. Regeneration with 1 mM HCl worked well and the frequency response in buffer after the regeneration step only started to increase after 15-20 hybridisation-regeneration cycles, probably because part of the coating was removed together with the bound analyte. Furthermore, the coated crystals could be kept at 4° for several weeks without losing their activity.

4.5. Comparison between the tested procedures

The evaluation of the immobilisation procedure from the four schemes tested, was achieved by considering the amount of streptavidin and biotinylated probe immobilised, the frequency shifts resulting from the hybridisation reaction and the extent of non-specific adsorption represented by the frequency shift resulting from the interaction between the probe and a non-complementary oligonucleotide.

In Figure 3 the results of the immobilisation of streptavidin and of the biotinylated probe are given for each of the four modification procedures. In the three that required the immobilisation of streptavidin, the frequency shifts resulting from this step were similar, and all demonstrated the suitability of the coating procedure for the immobilisation of the protein. The coefficient of variation (CV) of the method based on thiols and dextran was higher (~20%) compared to the other methods and this is probably due to the larger number of steps required for this coating scheme. The modification procedure based on thiols and dextran was more efficient, with the frequency shift resulting from immobilisation of the biotinylated probe being almost twice that for the other immobilisation schemes. Reproducibility was very similar for all the methods (~30%).

The hybridisation reactions between the immobilised probe and the complementary oligonucleotide for each of the immobilisation procedures are reported in Figure 2. Clearly, higher sensitivity was achieved by the use of thiols and dextran: the first two methods, the one without streptavidin and the one with mixed thiols and streptavidin, showed a very low sensitivity. The frequency shifts relative to the hybridisation with the probe immobilised with mercaptoundecanoic acid and streptavidin reached a plateau after 0.25 μM, whereas with thiols/dextran/streptavidin a linear increase with concentration increasing in the range 0.1-1.0 μM was obtained. The average

CV was much lower in the case of this last immobilisation procedure (16%) with respect to all the other three (>20%).

From Figure 4 we can make some comment on non-specific adsorption. The interaction of the immobilised probe with the non-complementary oligonucleotide resulted in a measurable frequency shift in the methods based on thiols or mixed thiols and streptavidin. In the other two cases no significant frequency decrease was recorded after this interaction. The number of hybridisation/regeneration cycles was much higher in the case of the immobilisation procedure based on thiols/dextran/streptavidin (Figure 5).

Considering the sensitivity, the amount of protein and probe immobilisation, the absence of non-specific adsorption and the possibility of regeneration, the immobilisation procedure based on thiols/dextran/streptavidin is recommended for the modification of the crystals for this application. This modification scheme requires about three days for the coating of the crystal with carboxylated dextran, but once modified, the crystal can be stored at +4°C for about one month. The immobilisation of streptavidin and of the probe is completed in about 1 h.

5. Conclusions

Four different modification procedures were tested for the realisation of a piezoelectric DNA biosensor. The biosensor can be applied to the determination of the hybridisation reaction. The immobilisation methods were used for the modification of the gold surface of piezoelectric crystal with oligonucleotide probes. The procedures were compared with respect to the measurement of hybridisation, the possibility of regeneration and non-specific adsorption. All the methods tested required the use of thiols. Three of them are based on the use of streptavidin-biotin interaction. The best results were obtained with dextran and streptavidin.

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Legends

Figure 1 Frequency variations during an affinity reaction taking place on the surface of the crystal. The analytical data are represented by the frequency differences between points A and B.

Figure 2 Sensitivity relative to the hybridisation reaction between the complementary oligonucleotide and the probe immobilised with: aminoethanethiol; 11-mercaptoundecanoic acid/streptavidin; mixed thiols/streptavidin; 11-mercaptoundecanol/dextran/streptavidin.

Figure 3 Comparison between the frequency shifts resulting from the immobilisation steps realised with the four modification procedures: aminoethanethiol; 11-mercaptoundecanoic acid/streptavidin; mixed thiols/streptavidin; 11-mercaptoundecanol/dextran/streptavidin.

Figure 4 Non-specific adsorption expressed as frequency shift for the interaction between the probe and a non-complementary oligonucleotide (1 μM), relative to the hybridisation reaction between the complementary oligonucleotide and the probe immobilised with: aminoethanethiol; 11-mercaptoundecanoic acid/streptavidin; mixed thiols/streptavidin; 11-mercaptoundecanol/dextran/streptavidin.

Figure 5 Number of hybridisation/regeneration cycles possible on the same quartz crystal modified with: aminoethanethiol; 11-mercaptoundecanoic acid/streptavidin; mixed thiols/streptavidin; 11-mercaptoundecanol/dextran/streptavidin.

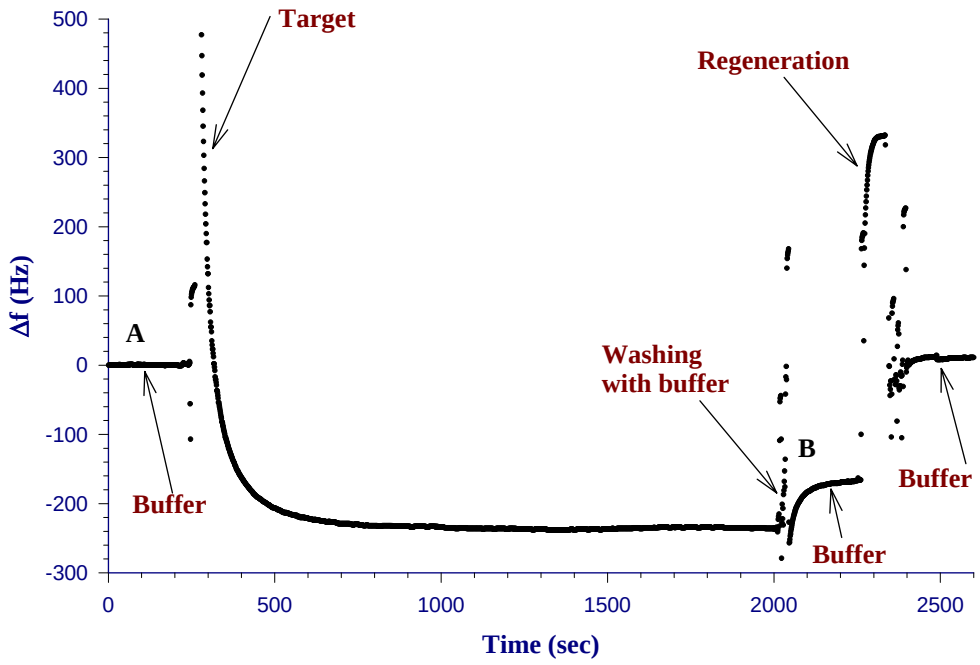
Target
Regeneration
A
Washing
with buffer
B
Buffer
Buffer
Buffer
Δf (Hz)
Time (sec)
500
400
300
200
100
0
-100
-200
-300
0
500
1000
1500
2000
2500

Figure 1

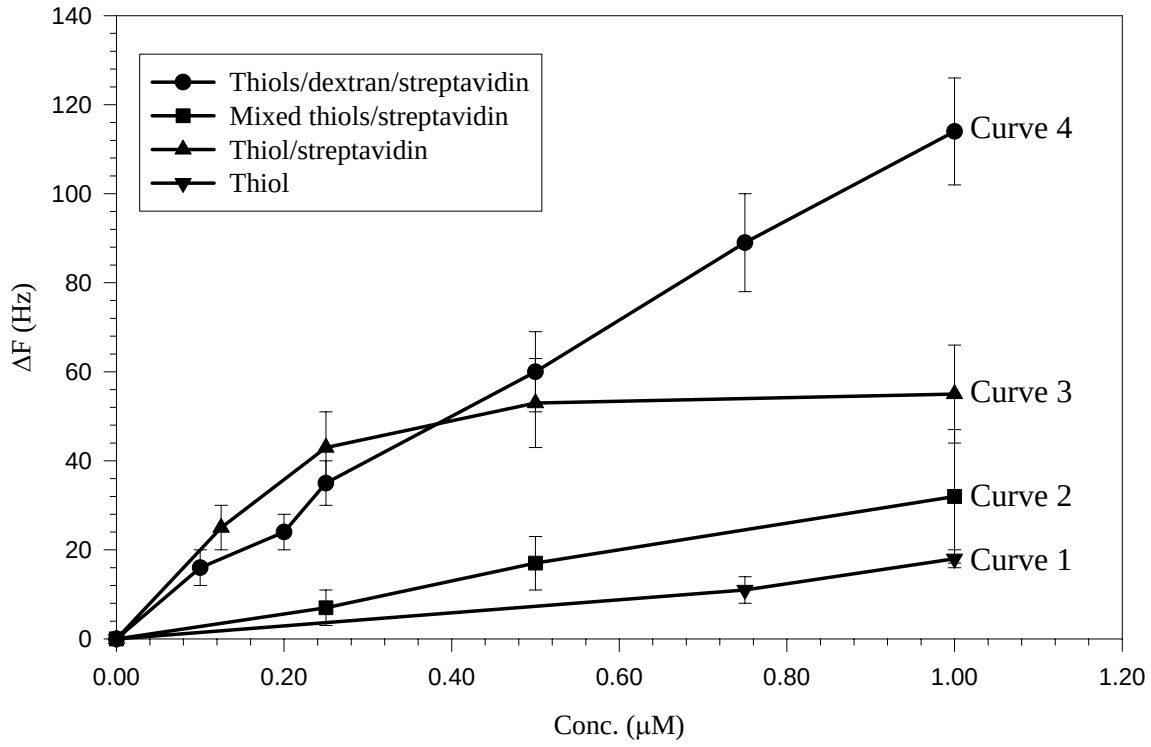
Thiols/dextran/streptavidin
Mixed thiols/streptavidin
Thiol/streptavidin
Thiol
Curve 4
Curve 3
Curve 2
Curve 1
ΔF (Hz)
Conc. (μM)
0
20
40
60
80
100
120
140
0.00
0.20
0.40
0.60
0.80
1.00
1.20

Figure 2

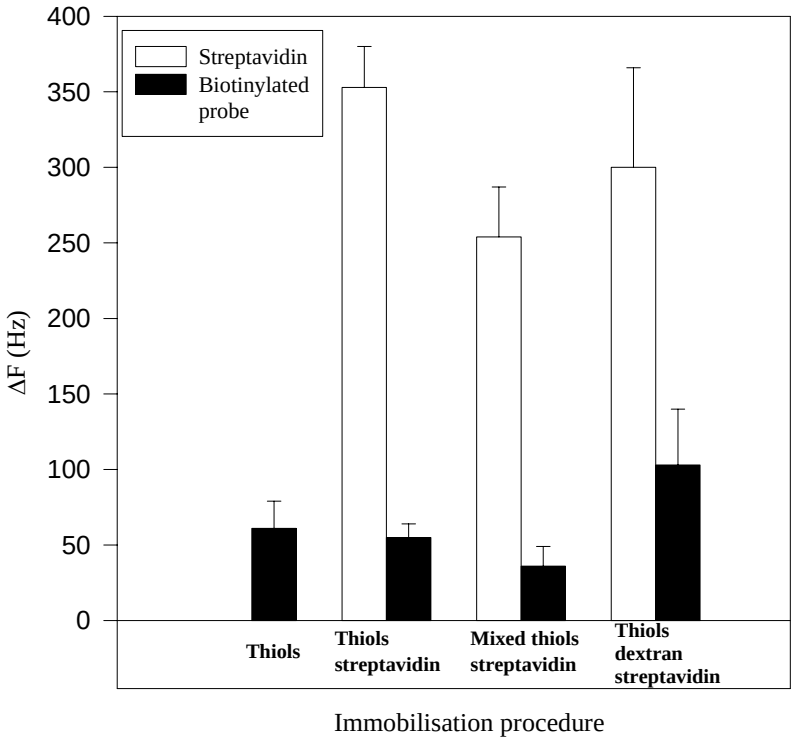
400
350
300
250
200
150
100
50
0
ΔF (Hz)
Streptavidin
Biotinylated probe
Thiols
Thiols streptavidin
Mixed thiols streptavidin
Thiols dextran streptavidin
Immobilisation procedure

Figure 3

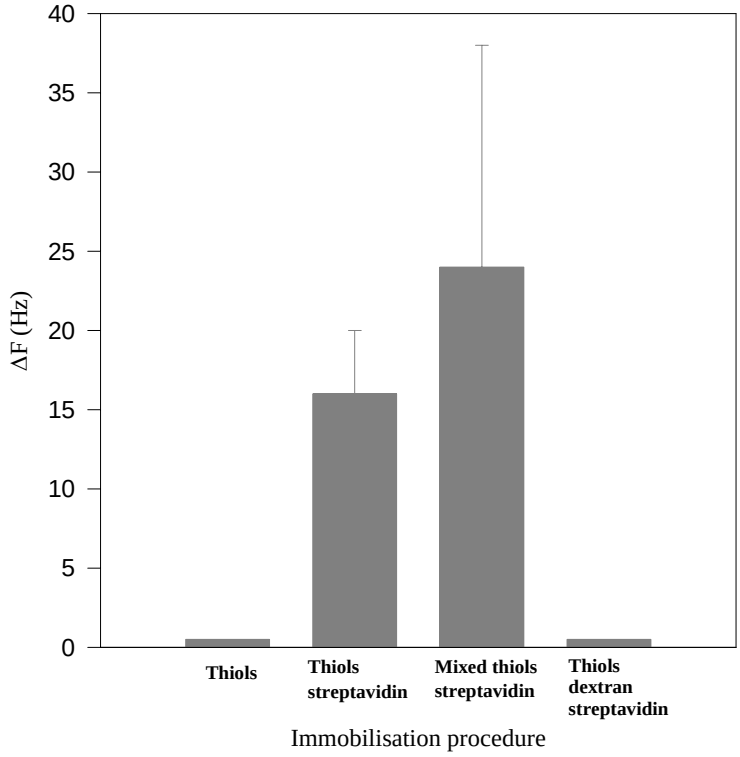
40
35
30
25
20
15
10
5
0
ΔF (Hz)
Thiols
Thiols streptavidin
Mixed thiols streptavidin
Thiols dextran streptavidin
Immobilisation procedure

Figure 4

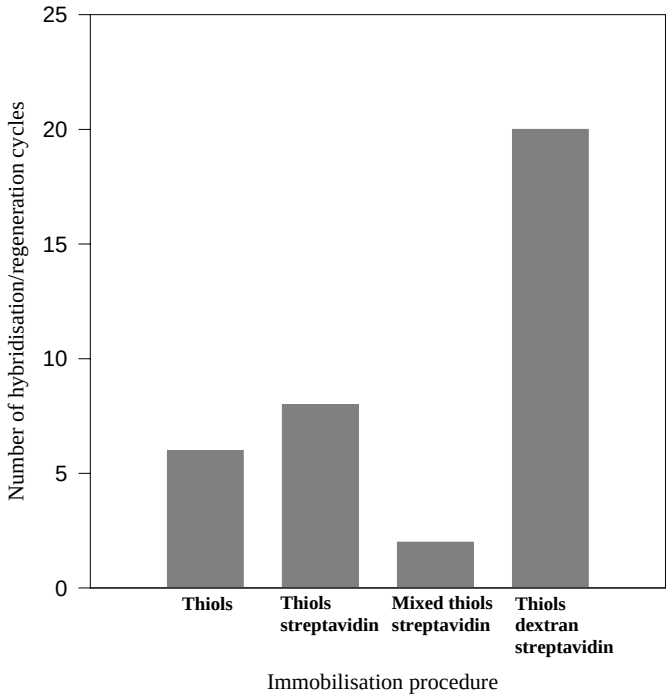
25
20
15
10
5
0
Number of hybridisation/regeneration cycles
Thiols
Thiols streptavidin
Mixed thiols streptavidin
Thiols dextran streptavidin
Immobilisation procedure

Figure 5