Coordination complexes as molecular glue for immobilization of antibodies on cyclic olefin copolymer surfaces

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A novel metal-based chelating method has been used to provide an order of magnitude increase in immunoassay performance on cyclic olefin copolymer (COC) plastics compared with passive binding. COCs are hydrophobic, and without surface modification they are often unsuitable for applications where protein adhesion is desired. When interacting with the bare plastic, the majority of the bound proteins will be denatured and become nonfunctional. Many of the surface modification techniques reported to date require costly equipment setup or the use of harsh reaction conditions. Here, we have successfully demonstrated the use of a simple and quick metal chelation method to increase the sensitivity, activity, and efficiency of protein binding to COC surfaces. A detailed analysis of the COC surfaces after activation with the metal complexes is presented, and the immunoassay performance was studied using three different antibody pairs.

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Cyclic olefin copolymers (COCs) are amorphous thermoplastic materials that have recently been used extensively for various applications such as biosensors [1,2], biodiagnostic chips, microfluidic devices [3,4], micro total analysis systems [5], DNA immobilization, immunoassays, and microarrays [6]. The clarity and optical resistance against commonly used sterilization regimes [7] makes COCs more attractive for many more applications than polystyrene (PS), which tends to cloud when sterilized with ethylene oxide or radiation. In addition, COCs offer high chemical resistance to acids, bases, and polar organic solvents (e.g., dimethyl sulfoxide, methanol, acetone) as well as thermal resistance by selecting COCs with high glass transition temperatures [8]. Furthermore, COCs are photosensitive, which allows the writing of gratings for selective and label-free biosensing [9]. In contrast to poly(methyl methacrylate) (PMMA), COCs are less sensitive to humidity [10] and have orders of magnitudes lower loss at terahertz frequencies [11]. The density of COCs is approximately half the density of glass, and COCs are less brittle, which makes them an attractive alternative to glass in optical components when weight or durability becomes important. By comparison, polycarbonate and PMMA, both used as alternative materials in life sciences, are brittle, are prone to chemical degradation, and have inferior optical properties compared with COCs [12].

Despite COCs offering excellent bulk properties for many applications as described above, surface modification of these materials remains a challenge. These thermoplastic materials are prepared by chain copolymerization of cyclic monomers such as norbornene with ethylene or by ring-opening methathesis polymerization of cyclic monomers followed by hydrogenation [13]. Their pure hydrocarbon composition means that they lack readily accessible functional groups. The surface hydrophobicity of COC promotes fouling by proteins, a complicating factor when adhesion of proteins is unwanted. In cases where adhesion of proteins is desired, nonspecific passive immobilization of proteins often leads to loss of function. The introduction of hydrophilic polar functional groups
through various surface pretreatments, to increase the surface free energy, has been suggested as a method to minimize such difficulties. Among the surface modification techniques reported on COCs are plasma treatment [3,14–17], ultraviolet (UV) [5], gamma or electron beam radiosterilization [18], and chemical treatment [19,20] to oxidize the nonreactive surface.

Typically during the surface modification process, the polymer surface is modified through oxidation, degradation, and cross-linking, which inevitably causes structural alterations to the first few molecular layers on the polymer surface. Plasma treatment derived from oxygen, ammonia, and noble gases is one of the commonly used surface pretreatment methods. Although COC surfaces are successfully rendered hydrophilic by the plasma, these surfaces are unstable because polymer chains on the surface tend to rearrange and return the surface to the native unreactive hydrophobic form. This phenomenon has been widely confirmed through increment in contact angle measurements [21] and ζ-potential decay [22], which often occurs over a period of days. Furthermore, the source of surface charge after pretreatment with plasma, gamma, or electron beam radiosterilization is unclear. To optimize the hydrophilic functionality and energy of COC surfaces, pretreatment for long duration and high power plasma source has been conducted. Although this may increase shelf life, the surface roughness can be compromised, thereby rendering the materials incompatible with applications that require homogeneous surface roughness.

It is well known from several decades of experience in enzyme-linked immunosorbent assay (ELISA) that plain PS microtiter plates without surface modification are suitable for only a small percentage of proteins. More hydrophilic proteins such as antibodies require polar groups on the plate surface, and as a consequence there are many types of surface-modified microwell plates. Following this learning, increasing the polarity of COC surfaces is likely to be essential to improve its ability to bind antibodies. The different types of cyclic olefin polymers (COPs) and COCs available commercially have varying abilities to bind proteins. Some workers have reported problems associated with nonspecific binding to COC surfaces [23]; however, there have also been reports of COP materials that are inert to biological molecules. Niles and Coassin reported the use of COP (Zeonor 1420R) as the base material for a high-density multiwell plate (Aurora Biotechnologies) [12]. These plates were reported to be inert to biological molecules except small hydrophobic polypeptides less than 10 kDa. Based on these reports, it is difficult to achieve desirable antibody binding properties on these surfaces without applying surface treatments.

Where passive binding is undesirable or covalent linkages are difficult to achieve, a completely different approach to binding proteins via the use of metal complexes can be used [24]. Using a high-throughput surface discovery approach, many thousands of metal complexes were screened for their efficiency to bind antibodies, and several particular complexes were found to be highly efficient in binding proteins. These particular metal complexes, called Mix&Go [25–27], depend on two basic characteristics for binding. Using slow exchanging metal complexes such as chromium(III) in oligomeric form, there is avidity or multicomponent chelation to the synthetic surface while retaining potential to similarly bind proteins. A single metal–ligand interaction may readily break; however, the odds of multiple interactions breaking simultaneously are low. However, COC plastics are hydrophobic and should not have electron donating groups to chelate to metal complexes.

It has been previously shown that versions of such metal complexes can bind to plain non-irradiated PS surfaces. Presumably, these metal complexes are in coordination with the π-electrons of the phenyl ring, and although one interaction may be weak, a multiplicity of interactions is sufficient to create a completely different surface over the previously hydrophobic nonfunctional surface. Considering their structure, COP/COC surfaces do not have electron donating potential to bind metal complexes. However, work on enhancing adhesion of metals onto such COC/COP surfaces has been reported [8,15]. Nikolova and coworkers studied the effects of plasma treatment on the adhesive strength of aluminum and copper on COC surfaces [15]. The adhesive strength in COC–metal composites increased with the intensity of plasma treatment; however, the untreated controls demonstrated the ability to bind these metals as well. If a weak potential to bind metals on COC surfaces exists, a basic strategy of using slowly exchanging metal complexes and avidity of such metals using polymeric metal constructs will potentially allow the formation of a surface that promotes protein binding. Once formed, the residual coordination sites remaining after forming metal complexed surfaces are chelated to small ligands such as water that undergo exchange with a specific half-life. In other words, the Mix&Go activated COC surfaces can be stored but remain active indefinitely. Importantly, in the presence of many classes of biomolecules (e.g., antibodies, streptavidin, protein A or G), coordination forces bind such proteins firmly onto the surface.

In this study, the potential of the metal complexes to bind to COC surfaces to form a hydrophilic chelating surface was investigated. Three different sandwich assays were performed using one particular formulation called Mix&Go Biosensor, which was known to perform well on PS surfaces as well as silica and other metal oxide surfaces. Two assays were known to work well by passive binding on PS microtiter plates, and the third sandwich assay gave poor results under the same conditions. To better understand the effects of metal coordination on such hydrophobic surfaces, surface analytical techniques such as X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM), and contact angle studies on these metal complex-activated surfaces were performed.

Materials and methods

Materials

2-[(N-Morpholino)ethanesulfonic acid (Mes), phosphate buffer saline (PBS), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes), bovine serum albumin (BSA), Tween 20 (T20), sulfuric acid, and Greiner Bio-One COC plates (cat. no. M3812) were obtained from Sigma–Aldrich. Troponin I (TnI) monoclonal antibody (MAB, 19C7), human cardiac TnI antigen, and biotinylated TnI MAB (16A11) were purchased from HyTest (Finland). MAB1 tumor necrosis factor alpha (TNFα) antibody, biotinylated MAB11 TNFα antibody, and streptavidin–horseradish peroxidase conjugate (Strep–HRP) were purchased from BD Biosciences. Recombinant human TNFα was purchased from R&D Systems (USA). Clone 057-1-1003 thyroid-stimulating hormone (TSH) MAB was purchased from Meridian Life Science. Clone 5403 biotinylated TSH MAB was purchased from Medix Biochemica. Goat anti-mouse–horseradish peroxidase conjugate (GM–HRP) was obtained from Jackson ImmunoResearch (USA). 3,3′,5′-tetramethylbenzidine (TMB) substrate was purchased from Thermo Scientific. Mix&Go Biosensor was obtained from Anteo Diagnostics. Buffers prepared were 50 mM Mes at pH 5.2 (coating buffer for TNFα and TSH assays), 10 mM PBS with 1% BSA (assay buffer for TNFα and TSH assays), 50 mM Mes with 5% BSA (blocker for TNFα and TSH assays), 10 mM PBS with 0.05% T20 (wash buffer for TNFα, TSH, and TnI assays), Hepes at pH 7.4 (coating buffer for TnI assay), 10 mM PBS with 0.1% T20 (assay buffer for TnI), and PBS with 1% casein (blocker for TnI assay).

Characterization

13C nuclear magnetic resonance (NMR) spectra were obtained on a Bruker AVANCE 400-MHz instrument operating at
400.13 MHz with a standard Bruker 5-mm broadband observed gradient probe. Deuterated chloroform (CDCl$_3$) was used as the solvent. Spectra were referenced to solvent signal at δ$_{CDCl_3}$ = 77.0 ppm. All spectra were processed using Bruker TOPSPIN.

Contact angle measurements were carried out on an apparatus composed of an adjustable stage and lens assembly fitted with a camera and linked to Scion imaging software. The sample was placed on the stage, and 5 μl of Milli-Q water was transferred by a 50-μl glass flat-tip syringe onto the sample surface. The needle tip was removed from the water droplet before capturing an image of the droplet. Contact angles were measured using the imaging software. All measurements were taken five times.

A Kratos Axis Ultra X-ray Photoelectron Spectrometer with monochromatic Al Kα X-rays (1486.6 eV) at 150 W (15 kV, 10 mA) was used to collect XPS spectra. Photoelectron data were collected at a takeoff angle of theta = 90°. Survey scans were taken at an analyzer pass energy of 160 eV. Survey scans were performed over a range of 1200 to 0 eV binding energies with 1.0-eV steps and a dwell time of 100 ms. The base pressure in the analysis chamber was set at 1.0 × 10$^{-9}$ Torr and during sample analysis was set at 1.0 × 10$^{-8}$ Torr. Processing was performed using CasaXPS. Binding energy corrections were made by referencing spectra to the carbon 1s fixed at 285 eV [28]. Survey scans were taken of an area of 0.7 × 0.3 mm$^2$.

An MFP-3D (Asylum Research) atomic force microscope was used for all of the measurements. The cantilevers used were HA_NC (Etalon) from NT-MDT (Russia) having a nominal spring constant of 4.5 N/m and a nominal resonant frequency of 145 kHz. All of the images were obtained by employing the tapping mode of the atomic force microscope in air. The microscope was mounted on an anti-vibrational table (Herzan) and operated within an acoustic isolation enclosure (TMC, USA).

Immunoassays

The plates were treated by incubating each well with Mix&Go Biosensor solution for 1 h. Wells were then washed thoroughly with deionized water, followed by a wash with coating buffer. For passive binding experiments, the plates were used as received.

For the loading assays, capture antibodies were diluted in coating buffer at 1 μg/ml and incubated in plates for 30 min before washing with wash buffer twice on a Tecan 96 Plate Washer. Plates were then blocked with blocking buffer for 1 h. The washing step was carried out three times to remove excess blocker. This was followed by the incubation of GAM–HRP in assay buffer (0.1 μg/ml) for 30 min and then washing five times with wash buffer. The substrate, TMB, was reacted with HRP for 7 min before the reaction was terminated by the addition of 2 M sulfuric acid, which resulted in a yellow product.

For the sandwich assays, capture antibodies were diluted in coating buffer at desired concentrations and incubated in plates for 30 min (unless stated otherwise) before washing with wash buffer twice on a Tecan 96 Plate Washer. Plates were then blocked with blocking buffer for 1 h. The washing step was carried out three times to remove excess blocker. Antigen in assay buffer was incubated in plates for 1 h before washing five times with wash buffer to remove excess analytes. This was followed by the incubation of biotinylated detection antibodies in assay buffer (0.5 μg/ml) for 30 min and then washing five times with wash buffer. Strep–HRP (0.1 μg/ml in assay buffer) was then reacted with the biotinylated antibodies for 15 min and washed five times with wash buffer. The substrate, TMB, was reacted with HRP for 7 min before the reaction was terminated by the addition of 2 M sulfuric acid, which resulted in a yellow product.

Optical density (OD) of the colorimetric substrate for immunoassays was measured using a Tecan Infinite 200 PRO.

The measurements were conducted at 450 nm with the reference wavelength at 620 nm. The bandwidth at both wavelengths was set to 9 nm. The temperature of the measurements was 23 or 24 °C.

Results and discussion

Chemistry of the surfaces

The class of commercial materials known as COCs encompasses a large number of potential cyclic monomers prepared using various polymerization methods. The chemical structure and composition of the COC polymers are important because they determine the physical and surface properties of the materials. Changes in the copolymer content and chemical structure of the cyclic monomers can modify physical properties such as microstructure, glass transition temperature, and surface energy [13,22,29]. Shin and coworkers reported comprehensive 13C NMR studies of the chemical structures of a series of commercial COCs and COPs and related these to the thermal and surface properties [13]. They demonstrated that with increasing amounts of bulky cyclic monomer units, the glass transition temperature of the copolymers increases and the presence of ester or ether groups increases the surface energy. The chemical composition of the COC plates used in these studies was determined by 13C NMR, as shown in Fig. 1, and the polymer was found to be composed of ethylene and norbornene monomers. The peaks in the NMR spectra were assigned according to the extensive NMR studies that have been reported in the literature [13,30,31]. Resonances from the ethane-1,2-diyl units (carbons 8 and 9) and the cyclic monomer (carbons 5 and 6) overlapped in the region between 29.8 and 31.8 ppm. Other peaks due to the norbornadienyl units are at 32.7 ppm (carbon 7) and between 40.7 and 41.6 ppm (carbons 1 and 4). The main peaks of interest lie in the region of 46 to 48 ppm and are assigned to the methine carbons (2 and 3) of the norbornadienyl units. These peaks provide information on the composition and structure of the copolymer. Rische and coworkers showed that, in general, COCs display either of two distinct NMR spectra depending on the norbornene content [32]. Well-separated and narrow peaks were observed when the fraction of norbornene in the COC used was less than 50%, as opposed to broader and less separated peaks for higher norbornene contents. These observations indicate that the COC studied here has less than 50% norbornene units and consists mainly of alternating ethenylidippedenedienyl and long sequences of ethylene units. NMR does not indicate the presence of oxygen or other groups having electron donating potential within this COC material.

The COC plates were treated with Mix&Go simply by exposing the surfaces to the metal polymer solution for 1 h under ambient conditions (M&G–COC). The presence of bound metal complexes on the COC surface was confirmed through the detection of chromium peaks by XPS. Fig. 2 shows the XPS spectra of the COC surfaces before and after treatment with Mix&Go. Measurements were conducted on three different areas of four treated wells, which were distributed randomly in a plate (see online Supplementary material). Table 1 shows the percentages of Cr relative to other elements measured in each well. The variability in the distribution of Cr between wells was relatively high, ranging from 0.43 to 2.67% in the four wells tested. The Cr complex was stable to multiple washing steps, indicating that by some mode of action the metal complexes could be bound to COC surfaces [8,15]. The untreated COC surface showed the presence of zinc, silicone, and oxygen, which may be attributed to the presence of molding agents and contaminants or possibly oxidation of the surface introduced during the injection molding process. It may be possible that these oxygen species are the anchoring points for avidity binding of
these metal oligomers. Once the metal complex is deposited on these sites, the complexes may aggregate or diffuse on the surface or through the bulk polymer bulk via random diffusion processes [33]. This may contribute to the variation in level of Cr detected in the wells by XPS.

The roughness of the COC surfaces before and after treatment with Mix&Go was measured by AFM, as shown in Fig. 3. On magnification (5 × 5 μm² area), the metal complexed COC surfaces looked distinctly different from the untreated surface, confirming that a metal complex film has coated onto the COC. The roughness of the COC surfaces was measured by AFM to be 4.7 ± 0.5 nm and was 4.5 ± 1.0 nm after treatment with Mix&Go. These results indicate that treatment with the metal complex solution did not alter the topography of the surfaces. In contrast, alternative surface treatments can significantly increase surface roughness. COC surfaces are commonly rendered hydrophilic through exposure to high-energy sources such as plasma and gamma irradiation. These treatments produce unstable hydrophilic surfaces and, over prolonged exposure time, surface etching occurs. Roy and coworkers observed an increase in roughness from 11 to 23 nm after a 120-s exposure to argon plasma treatment [3]. Further exposure up to 180 s produced COC surfaces with higher roughness, and the use of oxygen instead of argon produced even rougher surfaces. Similarly, Nikolova and coworkers observed an increase in roughness.

Table 1
Distribution of chromium on different COC wells.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Average % of Cr</th>
<th>Standard deviation</th>
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<tbody>
<tr>
<td>1</td>
<td>2.67</td>
<td>0.15</td>
</tr>
<tr>
<td>2</td>
<td>0.69</td>
<td>0.12</td>
</tr>
<tr>
<td>3</td>
<td>0.43</td>
<td>0.01</td>
</tr>
<tr>
<td>4</td>
<td>1.32</td>
<td>0.24</td>
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roughness from 4.3 to 8.2 nm on their COC surfaces after 60 s of oxygen plasma treatment [15].

The hydrophilicity of the COC surfaces was also determined via contact angle measurements. The contact angle of the hydrophobic COC surface was measured to be 95.6 ± 3.9°, which was similar to previously reported values [5]. The treatment with Mix&Go did not significantly alter the hydrophobicity of the COC surfaces given that the contact angle was found to be 104.5 ± 2.6°. The detection of chromium and the underlying surface using XPS and the minimal changes in roughness in the AFM images are suggestive that the metal complex coatings are very thin films and might not totally cover the underlying surface. The absence of significant changes in the contact angle further indicates that the metal complex coating applied was insufficient to alter the hydrophilicity of the surfaces. This shows that the application of Mix&Go retains the original properties of the COC surfaces but is adequate to provide superior improvements to the immunological performance of the materials, as discussed in the following section.

### Immunological performance

Binding of biomolecules such as antibodies and other proteins on untreated COC surfaces is driven by hydrophobic interactions, which often lead to conformational damage and subsequent loss of functionality. From the surface analysis studies, we have shown that treatment with Mix&Go has created a very thin film of metal complexes that might not totally cover the underlying hydrophobic surface (by contact angle measurements). This is consistent with the lack of electron donating groups on the COC surface, leading to a new surface that may still include the potential for hydrophobic binding but now augmented by metal coordination forces. This could be likened to PS microtiter plates, which have been surface treated to improve passive binding of more hydrophilic proteins such as antibodies.

To test antibody binding performance on COC plates treated with Mix&Go, three sandwich ELISAs (TNFα, TnI, and TSH) were selected. The TNFα antibody pair was recommended for ELISA by the manufacturer. The TSH antibody pair has been confirmed by antigen binding on untreated and Mix&Go-treated COC surfaces. The detection of chromium and the underlying surface using XPS and the minimal changes in roughness in the AFM images are suggestive that the metal complex coating applied was insufficient to alter the hydrophilicity of the surfaces. This shows that the application of Mix&Go retains the original properties of the COC surfaces but is adequate to provide superior improvements to the immunological performance of the materials, as discussed in the following section.

![AFM images of untreated COC (A) and M&G–COC (B) surfaces.](image)

**Fig. 3.** AFM images of untreated COC (A) and M&G–COC (B) surfaces.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Loading (IU/ml)</th>
<th>Sensitivity (OD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFα</td>
<td>1000</td>
<td>0.43-0.76</td>
</tr>
<tr>
<td>TnI</td>
<td>1000</td>
<td>0.43-0.76</td>
</tr>
<tr>
<td>TSH</td>
<td>5</td>
<td>0.43-0.76</td>
</tr>
</tbody>
</table>

The results of sandwich ELISAs (1000 pg/ml TNFα, TnI, and TSH antigen) at the same capture antibody concentration are shown in Fig. 4B. The amount of functional antibodies, as evidenced by antigen binding on untreated and Mix&Go-treated COC surfaces, clearly shows increased assay performance under the experimental conditions. Both TnI and TSH assays did not give any signal with passive binding, whereas TNFα assays on Mix&Go-treated surfaces gave approximately nine times greater signal.

To further understand the differences between untreated and Mix&Go-activated COC surfaces, titrations of capture antibody as well as antigens for all three ELISAs were also conducted. Fig. 5 shows the titration curves for the TNFα system. Capture antibody titration was conducted at an antigen concentration of 1 μg/ml, TNFα and TnI antibodies on untreated and Mix&Go-treated COC surfaces were different, as determined by anti-species antibody binding and consistent with the differences observed by surface analysis. Using a capture antibody concentration of 1 μg/ml, TNFα and TnI antibodies can be successfully bound to untreated COC surfaces by passive binding. However, the TSH antibody either did not bind or was totally damaged such that it could not be detected by the anti-species antibody. In contrast, there was an increase in loading for all three capture antibodies on Mix&Go-treated COC surfaces. However, the absolute loading was different for each antibody, suggesting some antibody-specific factors in this loading assay.

The results of sandwich ELISAs (1000 pg/ml TNFα and TnI antigen and 5 μIU/ml TSH antigen) at the same capture antibody concentration are shown in Fig. 4B. The amount of functional antibodies, as evidenced by antigen binding on untreated and Mix&Go-treated COC surfaces, clearly shows increased assay performance under the experimental conditions. Both TnI and TSH assays did not give any signal with passive binding, whereas TNFα assays on Mix&Go-treated surfaces gave approximately nine times greater signal.

TNFα sensitivity on the M&G–COC surfaces was significantly better, implying that the functionality of capture antibodies on
M&G–COC was maintained, whereas passive binding on untreated COC must have led to functional damage. Similar improvements were also observed for the TnI and TSH sandwich assays. The TSH capture antibody titration curve reached a plateau earlier on the M&G–COC surfaces at approximately 5 µg/ml, whereas the passive curve increased up to the concentrations studied (Fig. 6A). There was a clear antigen titration on the M&G–COC surface, whereas the same assay on the untreated surface failed (Fig. 6B). Similarly, the TnI assay (Fig. 7) required fewer antibodies to reach a maximum with higher antibody loading capacity and obtained a measurable antigen titration on the M&G–COC surfaces compared with the untreated COC surfaces. It should be noted that the measured OD values for the TnI assays are relatively low, but this is not surprising considering that this capture antibody was known to be sensitive to passive binding. TnI sandwich assays using this particular capture antibody on commercially available PS microtiter plates (MaxiSorp and Greiner Bio-One) gave very poor performance (see Supplementary material). Interestingly, on both treated and untreated surfaces, there were relatively large quantities of immobilized antibodies by loading assay, indicating that the poor ELISAs were likely due to degradation of the TnI antibodies.

In addition to the improved assay performance, the binding of antibodies to M&G–COC surfaces occurred at a much faster rate compared with passive binding, suggesting a completely different mode of binding. Typically, antibody coating on PS plates requires an overnight incubation [34]. Fig. 8 shows the coating capture antibody time profiles for the three sandwich assay systems. On the M&G–COC surfaces, a dramatic increase occurs during the first 10 min of incubation for all three systems. As expected, the passive COC surfaces required longer incubation periods for antibodies to fully coat the wells. To demonstrate the efficiency of M&G–COC over untreated COC surfaces, a 24-h incubation time point, which is considered to be the commonly used incubation period for
passive binding, was also measured for all three systems and is reported in Fig. 9. The measured signals after 24 h show that the increased incubation period on untreated COC did not improve the activity of the assays and was incapable of reaching the efficiencies demonstrated by the M&G–COC surfaces. The lower OD values measured for the 24-h time points compared with the shorter time points may be attributed to the deterioration of the biomolecules over time and the different kinetics observed at 4 °C.

**Conclusions**

In this study, a metal chelation system called Mix&Go Biosensor was tested on COC plastics, a surface that was expected not to have electron donating groups for coordination of metal ions. However, surface analysis clearly shows effective binding of these metal complexes to the surface by simple addition of these aqueous solutions to the wells of the COC microtiter plate followed by incubation for 1 h. These surface treatments were stable in their activated form and did not lead to any measurable change in surface roughness or hydrophobicity. For the three test assays, this treatment led to significant increases in sensitivity, capture antibody savings, and improved inter-well reproducibility to the point where it enabled assays that were not possible by passive coating.
COCs are highly hydrophobic and are known to lead to non-specific binding of proteins, which often render them nonfunctional. However, their superior optical properties over other commonly used polymers have increased their popularity in various applications, and where needed different surface modification techniques have been applied to change the surface properties in order to improve binding of proteins. Methods based on covalent binding are usually preferred because they are more controllable and minimize protein denaturation. However, covalent binding often involves syntheses that require synthetic skills. The method of surface treatment with Mix&Go implemented here is simple and economical and does not require in-depth chemistry laboratory skills or the use of any sophisticated instruments. This preliminary study did show variations in the amount of metal complexes in individual wells of a microtiter plate detected by XPS. However, these variations were not reflected in the immunoassay performance of the activated COC surfaces. The improved performance of M&G-COC compared with COC was demonstrated through assays conducted on two antibody pairs (TNFα and TSH) known to work on standard PS microtiter plates (by passive coating) and another antibody pair (cardiac biomarker, TnI) known not to work on standard PS microtiter plates (by passive coating). All of these capture antibodies have been shown to bind much faster and reach maximum coating at lower antibody concentrations, indicating minimal functional damage.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jab.2014.03.023.

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