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(54) Title: METHOD OF PROTECTING A SURFACE FROM BIOLOGICAL FOULING

(57) Abstract: The present invention relates to a method of protecting a surface from biological fouling. The present invention finds particular application in the protection of the surface of objects that are used in aquatic environments, particularly marine environments. The present invention also relates to kits for use in the methods of the present invention.

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METHOD OF PROTECTING A SURFACE FROM BIOLOGICAL FOULING

FIELD OF THE INVENTION

The present invention relates to a method of protecting a surface from biological fouling. The present invention finds particular application in the protection of the surface of objects that are used in aquatic environments, particularly marine environments. The present invention also relates to kits for use in the methods of the present invention.

BACKGROUND OF THE INVENTION

Biological fouling of surfaces is a constant problem in the modern world and is almost endemic as it can be observed in almost every environment into which man has spread. Examples of biological fouling include the growth of mosses and lichen on structures such as buildings, bridges and the like, the growth of mould in buildings and the fouling of objects that come into contact with aquatic environments such as boats, pilings and the like.

Biological fouling of man-made surfaces by aquatic organisms is a significant economic and environmental issue. Colonization of surfaces exposed to aquatic environments by bacteria, protists (e.g., diatoms and *Ulva*), fungi, tubeworms, sponges, barnacles, mollusks (e.g., clams, oysters, periwinkles and mussels) and other marine organisms can lead to deterioration of the surface coating, necessitating frequent re-application, and having a significant aesthetic impact. Where the coated surface is on a mobile vessel (such as a ship's hull), biological fouling can lead to significant increases in drag and, therefore, reduced fuel efficiency. Biological fouling also increases the downtime for commercial vessels, as repeated repainting becomes required. As well as having an impact on the cost of running a vessel, biological fouling also impacts on the efficiency of the entire shipping industry, with significant reductions in ship speed. Further, fuel efficiency reductions also have a substantial impact in the area of greenhouse gas production, as combustion of the additional fuel required to overcome drag caused by fouling organisms leads to increased CO₂ emissions. Biofouling also results in the importation of pest organisms that foul ship hulls and are then transported and deposited around the world, causing severe risks to native marine habitats the world over. There exists a need for a relatively inexpensive, durable, non-toxic antifoul coating.

Historically, the issue of aquatic biological fouling has been addressed by the incorporation into the coating of biocidal compounds, and in particular of organotin compounds such as tributyl tin and tributyl tin oxide (see, for example, US 4,497,852 and US 4,576,838). These compounds are effectively toxic to most biofouling species, but, as might

be imagined, can have obvious and highly deleterious side effects. In harbours where there is significant maritime traffic, these compounds (which leach out of the coating over time), can accumulate to alarmingly high levels, and moreover tend to bioaccumulate in the food chain. Ultimately, these compounds affect not only marine but human communities centered on such harbours. A move away from organotin compounds has been necessitated.

More recently, copper compounds such as copper oxide have been used extensively in marine coatings (see, for example, US 5,302,192). While less toxic than organotin compounds, these compounds also tend to accumulate to unsatisfactorily high levels in areas of heavy maritime traffic. Moreover, while effective against some of the larger fouling organisms, such as barnacles, the copper oxide based antifouling paints have never been as effective against fouling by bacteria and some protists, particularly diatoms and the green seaweed *Ulva* that often dominate biofilms. Therefore, there exists a real need for the development of antifouling surfaces that do not leach inorganic toxins into the marine environment, and which are effective against a wide variety of aquatic fouling species.

US Application 20070129461 discloses the use of a polymeric matrix incorporating an ionic monomer, wherein the ionic monomer is associated with an organic cationic biocide. In particular, the cationic biocide used is selected from among the polysubstituted cationic guanidinium derivatives, quaternary ammonium cations and pyridinium derivatives. While representing a move away from inorganic biocidal compounds, this invention nevertheless involves the use of toxic chemical species.

US 5,218,059 discloses the use of a non-toxic antifouling paint based on silicone resin. In this case, a certain proportion of non-curable silicone is incorporated into the coating, with the effect that the non-curable molecules eventually leach to the surface where they disrupt the interaction of biofouling organisms, thereby facilitating foul release.

US 6,265,515 discloses the use of a fluorinated silicone elastomer in the production of antifouling surfaces. The use of the fluorinated polymer is aimed at the production of a highly hydrophobic surface, in which the non-polar fluorine sites prevent the formation of hydrogen bonds with the biopolymer adhesive secreted by biofouling organisms, thereby resisting colonization by biofouling organisms. US 2003/0113547 also discloses the use of fluorinated surfaces in the reduction of marine biofouling. In this case, the fluorinated surface is derived from the incorporation of a fluorinated polyol into a polyurethane.

US Application 20070258940 discloses the application of cellulose esters in the preparation of toughened hydrophilic surfaces which resist microbial adhesion. In this case, the application of the ester is such that the formation of pores and imperfections in the surface is minimized. Crosslinkers are employed so as to ensure that there is appropriate stability for the coatings on items which are submerged in water.

US 6,241,898 discloses the use of a combination of surfactants (poly(ethylene oxide-co-propylene oxide) and various alkylsulfosuccinates) to block the adhesion of biofouling organisms to surfaces. This approach requires the addition of the surfactants to the process stream (which are then *free in solution*, rather than attached to the surface *per se*). While this approach is effective in closed systems, it is obviously not a practicable method for reducing the attachment of organisms to ocean going vessels and marine structures and devices.

US 6,039,965 discloses the application of block copolymers incorporating ethylene oxide units in solution as a means of preventing microbial adhesion to surfaces. In particular, this document disclosed the prevention of bacterial adhesion inside closed systems, for example, in a cooling system or in a pulp and paper making facility. Other aspects discussed include the prevention of microbial adhesion onto plastic surfaces.

WO 99/01514 discloses the application of polymers which have a broad spectrum anti-fouling capability derived from the incorporation of isothiazolones and/or furanones. These compounds were found to have an anti-fouling capability at very low concentrations of released compound. Moreover, the degradability of these compounds make them a preferable alternative to toxic inorganics. Nevertheless, the expense of incorporating "boutique" compounds such as furanones (some of which are derived from Australian red seaweed *Delisea pulchra*) into a commodity such as marine paint is a significant limitation.

There exists a need for a method of protection of a surface from biological fouling utilising components which are non-toxic, relatively inexpensive and relatively durable.

The discussion of the background to the invention herein is included to explain the context of the invention. This is not to be taken as an admission that any of the material referred to was published, known or part of the common general knowledge as at the priority date of any of the claims.

Throughout the description and claims of the specification the word “comprise” and variations of the word, such as “comprising” and “comprises”, is not intended to exclude other additives, components, integers or steps.

5 SUMMARY OF THE INVENTION

The present invention has arisen from the inventors' discovery that the surface of an object can be protected from biological fouling by applying to the surface an anti-fouling composition containing a polymeric material, the polymeric material (i) having side chains containing polymeric moieties that inhibit biological fouling. It has been found that such anti-fouling compositions are most efficiently applied by providing a first functional group on the surface of the object and incorporating a second functional group on the polymeric material in the antifouling composition and applying the anti-fouling composition to the surface of the object under conditions suitable for forming a bond between the first functional group and the second functional group.

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The present applicants have found that the presence of a polymeric moiety that inhibits biological fouling in the side chain(s) of the polymeric material of the anti-fouling composition to be particularly useful in anti-fouling applications. Without wishing to be bound by theory it is thought that as the side chain projects from the coating surface this serves to create a buffer zone of anti-fouling agent around the surface leading to improved anti-fouling properties. In addition the presence of the anti-fouling moiety in the side chain increases the overall density of anti-fouling agent in the polymeric material.

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There are a number of other potential benefits of the present system which incorporates the polymeric moiety that inhibits biological fouling in the side chain(s) of the polymeric material of the anti-fouling composition. In most applications the final anti-fouling properties of a treated surface will only be determined by the anti-fouling properties of the outer layer. This limits the application of compositions of this type as it requires the anti-fouling layer to be the outer layer and the protection provided by the anti-fouling composition decreases over time as the outer layer degrades and is slowly removed from the surface.

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In the present invention, as the polymeric moiety that inhibits biological fouling is located in the side chains of the polymeric material, the anti-fouling layer need not necessarily be the outer layer. This is because in circumstances where a further layer is added to a layer formed from the anti-fouling composition the side chains can penetrate or poke through the further layer leading to the effect that at least a portion of the polymeric moieties that inhibit biological fouling will be present on or projecting from the surface of the treated article even if

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the layer containing these materials is not the outer layer. This has the net effect that the anti-fouling property of the treated object will be more resilient to degradation as the layer containing the anti-fouling agent is harder to remove from the object (not being an outer layer).

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In addition in circumstances where there are a plurality of layers of polymeric material containing polymeric moieties that inhibit biological fouling the effect of the inhibitor being present in the side chains as discussed above is enhanced. Thus for example where there are three layers containing side chains containing polymeric moieties that inhibit biological
10 fouling the final surface of the object will be expected to contain side chains derived from each of the layers (as side chains from underlying layers will poke through or penetrate the layers above them) such that the total anti-fouling effect for the finally treated object is greater than that which could be achieved from a single application of the anti-fouling composition.

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In one aspect the present invention provides a method of protecting a surface of an object from biological fouling, the method including

- (a) providing a first functional group on the surface of the object;
- (b) providing an anti-fouling composition containing a polymeric material, the polymeric material (i) containing side chains of polymeric moieties which inhibit
20 biological fouling and (ii) containing a second functional group;
- (c) applying the anti-fouling composition to the surface of the object under conditions suitable for forming a bond between the first functional group and the second functional group.

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In some embodiments, the polymeric moieties which inhibit biological fouling include ethylene oxide units or vinylpyrrolidone units. In some embodiments, the polymeric moieties which inhibit biological fouling include ethylene oxide units.

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In some embodiments of the invention, the steps of the method may be repeated from 1 to 100 times. In some specific embodiments of the invention, the steps of the method may be repeated from 1 to 10 times. In some specific embodiments of the invention, the steps of the method may be repeated from 1 to 5 times. In some embodiments the steps of the method may be repeated once. In some other embodiments the steps of the method may be repeated twice. In still other embodiments, the steps of the method may be repeated three
35 times. In still other embodiments, the steps of the method may be repeated four times. In still other embodiments, the steps of the method may be repeated five times.

The step of providing the first functional group on the surface of the object may be carried out using any method known in the art. In some embodiments of the invention providing a first functional group on the surface of the object includes activation of the surface. In some embodiments activation of the surface includes oxidation of the surface. In some other embodiments activation of the surface includes acidification of the surface. In yet an even further embodiment activation of the surface includes reaction of the surface with a chemical entity to provide a first functional group on the surface.

In some embodiments of the invention providing a first functional group on the surface of the object includes applying to the surface a primer composition which includes a material containing the first functional group.

Accordingly in some specific embodiments the invention provides a method of protecting a surface of an object from biological fouling, the method including:

- (a) applying a primer composition including a material containing a first functional group to the surface of the object;
- (b) providing an anti-fouling composition containing a polymeric material, the polymeric material (i) having side chains containing one or more ethylene oxide units and (ii) containing a second functional group;
- (c) applying the anti-fouling composition to the surface of the object under conditions suitable for forming a bond between the first functional group and the second functional group.

In some embodiments of the invention, the steps of the method may be repeated from 1 to 100 times. In some specific embodiments of the invention, the steps of the method may be repeated from 1 to 10 times. In some specific embodiments of the invention, the steps of the method may be repeated from 1 to 5 times. In some embodiments the steps of the method may be repeated once. In some other embodiments the steps of the method may be repeated twice. In still other embodiments, the steps of the method may be repeated three times. In still other embodiments, the steps of the method may be repeated four times. In still other embodiments, the steps of the method may be repeated five times.

In the methods of the invention the primer composition may contain any suitable material that contains the first functional group. In some embodiments the primer composition contains a polymeric material containing the first functional group.

In some embodiments of the method of the invention the primer composition contains a polymeric material containing hydroxy groups. In some specific embodiments the primer composition contains poly(vinyl alcohol) as such a polymeric material.

5 In some embodiments the primer composition for use in the methods of the invention contains a polymeric material containing a positively charged species selected from the group consisting of protonated primary amino, protonated secondary amino, protonated tertiary amino and quaternary ammonium groups, or a negatively charged species selected from the group consisting of carboxylate groups, sulfonate groups, sulfate groups and phosphonate
10 groups.

In some specific embodiments the primer composition for use in the methods of the invention contains a polymeric material containing a positively charged species selected from the group consisting of protonated primary amino, protonated secondary amino, protonated
15 tertiary amino and quaternary ammonium groups. An example of a primer composition of this type is a primer composition that contains a polyamine. The polyamine may be any suitable polyamine although in some specific embodiments the polyamine is selected from the group consisting of poly(ethyleneimine), poly(allylamine hydrochloride) and poly(vinylamine hydrochloride).

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In some specific embodiments the primer composition for use in the methods of the invention contains a polymeric material containing a negatively charged species selected from the group consisting of carboxylate groups, sulfonate groups, sulfate groups and phosphonate groups.

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In some embodiments the primer composition for use in the methods of the invention contains a block, gradient, star or random copolymer of an acrylate with pendant azide groups and a polymerisable ester monomer of poly(ethylene glycol), or a block, gradient, star or random copolymer of an acrylate with pendant alkyne groups and a polymerisable ester
30 monomer of poly(ethylene glycol). In some specific embodiments the primer composition for use in the methods of the invention is a random copolymer. The random copolymer may be any suitable random copolymer although in some embodiments the primer composition contains a random copolymer of an acrylate with pendant azide groups and a polymerisable ester monomer of poly(ethylene glycol). In some other embodiments the primer composition
35 contains a random copolymer of an acrylate with pendant alkyne groups and a polymerisable ester monomer of poly(ethylene glycol).

In some embodiments of the methods of the invention the polymeric material in the anti-fouling composition contains epoxide groups. In some specific embodiments the epoxide groups are located in side chains of the polymeric material in the anti-fouling composition. In some more specific embodiments the polymeric material in the anti-fouling composition is a copolymer of glycidyl acrylate and/or glycidyl methacrylate and a polymerisable ester monomer of poly(ethylene glycol).

In some embodiments of the invention the anti-fouling composition contains a polymeric material containing a positively charged species selected from the group consisting of protonated primary amino, protonated secondary amino, protonated tertiary amino and quaternary ammonium groups, or a negatively charged species selected from the group consisting of carboxylate groups, sulfonate groups, sulfate groups and phosphonate groups.

In some embodiments the anti-fouling composition contains a polymeric material containing a positively charged species selected from the group consisting of protonated primary amino, protonated secondary amino, protonated tertiary amino and quaternary ammonium groups.

In some other embodiments the anti-fouling composition contains a polymeric material containing a negatively charged species selected from the group consisting of carboxylate groups, sulfonate groups, sulfate groups and phosphonate groups.

In some embodiments the anti-fouling composition contains a copolymer prepared by polymerization of a polymerisable ester monomer of poly(ethylene glycol) and a sulfonated ethylenically unsaturated monomer. In some specific embodiments the sulfonated ethylenically unsaturated monomer is 4-styrene sulfonate. In some specific embodiments the anti-fouling composition contains a block copolymer of sodium 4-styrenesulfonate and poly(ethylene glycol) methyl ether acrylate.

In some other embodiments of the invention the anti-fouling composition contains a random copolymer of an acrylate with pendant alkyne groups and a polymerisable ester monomer of poly(ethylene glycol).

In some other embodiments of the invention the anti-fouling composition contains a random copolymer of an acrylate with pendant azide groups and a polymerisable ester monomer of poly(ethylene glycol).

In many of the embodiments described above the composition (either primer or anti-fouling) contains a copolymer formed by polymerisation of a polymerisable ester monomer and another monomer. In essence a wide range of polymerisable ester monomers may be employed in the present invention although in some embodiments the polymerisable ester monomer is selected from the group consisting of poly(ethylene glycol) acrylates, poly(ethylene glycol) methacrylates, poly(ethylene glycol) methyl ether acrylates and poly(ethylene glycol) methyl ether methacrylates.

As stated above the anti-fouling composition can contain a polymeric material having side chains which inhibit biological fouling. In some embodiments the polymeric material has side chains containing one or more ethylene oxide units. In some embodiments each side chain contains between 1 and 1000 ethylene oxide units. In some other embodiments each side chain contains between 1 and 100 ethylene oxide units. In still other embodiments each side chain contains between 1 and 20 ethylene oxide units. In still other embodiments each side chain contains between 3 and 12 ethylene oxide units.

The methods of the present invention may be applied to the surface of a number of objects. In some embodiments the object is a ship, boat, yacht, dinghy, submarine, ferry, speedboat, frigate, aircraft carrier, minesweeper, ice breaker, rowboat, skiff, ketch, yawl, sloop, schooner, tanker, barge, or tugboat. In other embodiments the object is a surfboard, boogie board, windsurfer or waterski. In yet further embodiments the object is a buoy, pier, wharf, jetty, beacon, breakwater, oil rig, fish farm fence, shark net, fishing net, pontoon, crab pot, lobster pot, shark cage or dock. In yet even further embodiments the object is a keel, fin, mast, sail, rudder, propeller, periscope or cabin.

In certain other embodiments of the invention the object to be protected by the methods of the invention are the materials and equipment used in industrial processing. Of particular suitability for protection by the methods of the invention include those materials and equipment which are in constant, frequent or intermittent contact with aqueous solutions. Typical materials and equipment include those which are used in systems for power generation, water treatment, desalinisation and food processing. The materials and equipment may be found in power generation stations, water treatment plants, desalinisation plants and food processing plants, or any other factory wherein aqueous solutions are used. Materials suitable for protection by the methods of the invention include membranes and filters, for example reverse osmosis membranes and particulate filters. Typical equipment suitable for protection by the methods of the invention may include water intakes, piping systems, condensers, evaporators, valves, inlets, outlets and heat exchangers.

Other materials and equipment suitable for protecting by the methods of the invention include those used in small scale water treatment and desalination apparatus. Such materials include membranes and filters, for example reverse osmosis membranes and particulate filters. Typical equipment may include water intakes, piping systems, condensers, evaporators, valves, inlets, outlets and heat exchangers.

In a further aspect the invention provides an object containing a surface protected by a method as described above.

In yet an even further aspect the invention provides a kit for use in protecting a surface from biological fouling the kit including:

- (a) a primer composition including a material containing a first functional group;
- (b) an anti-fouling composition containing a polymeric material, the polymeric material (i) containing side chains of polymeric moieties which inhibit biological fouling and (ii) containing a second functional group;

wherein the first functional group and the second functional group form a bond once the two compositions have been applied to the surface.

In some embodiments of the kit, the polymeric moieties which inhibit biological fouling include ethylene oxide units or vinylpyrrolidone units. In some embodiments the polymeric moieties which inhibit biological fouling include ethylene oxide units.

In some embodiments of the kit the primer composition contains a polymeric material containing the first functional group. In some embodiments of the kit the first functional groups are hydroxy groups. In some specific embodiments of the kit the polymeric material containing the first functional group is poly(vinyl alcohol).

In some embodiments of the kit the primer composition contains a polymeric material containing a positively charged species selected from the group consisting of protonated primary amino, protonated secondary amino, protonated tertiary amino and quaternary ammonium groups, or a negatively charged species selected from the group consisting of carboxylate groups, sulfonate groups, sulfate groups and phosphonate groups.

In some specific embodiments of the kit the primer composition contains a polymeric material containing a positively charged species selected from the group consisting of protonated primary amino, protonated secondary amino, protonated tertiary amino and

quaternary ammonium groups. An example of a primer composition of this type is a primer composition that contains a polyamine. The polyamine may be any suitable polyamine although in specific embodiment the polyamine is selected from the group consisting of poly(ethyleneimine), poly(allylamine hydrochloride) and poly(vinylamine hydrochloride).

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In some specific embodiments of the kit the primer composition contains a polymeric material containing a negatively charged species selected from the group consisting of carboxylate groups, sulfonate groups, sulfate groups and phosphonate groups.

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In some embodiments of the kit the primer composition contains a block, gradient, star or random copolymer of an acrylate with pendant azide groups and a polymerisable ester monomer of poly(ethylene glycol), or a block, gradient, star or random copolymer of an acrylate with pendant alkyne groups and a polymerisable ester monomer of poly(ethylene glycol). In some specific embodiments the primer composition for use in the methods of the invention wherein copolymer is a random copolymer. The random copolymer may be any suitable random copolymer although in some embodiments the primer composition contains a random copolymer of an acrylate with pendant azide groups and a polymerisable ester monomer of poly(ethylene glycol). In other embodiments the primer composition contains a random copolymer of an acrylate with pendant alkyne groups and a polymerisable ester monomer of poly(ethylene glycol).

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In some embodiments of the kits of the invention the polymeric material in the anti-fouling composition contains epoxide groups. In some specific embodiments the epoxide groups are located in side chains of the polymeric material in the anti-fouling composition. In some more specific embodiments the polymeric material in the anti-fouling composition is a copolymer of glycidyl acrylate and/or glycidyl methacrylate and a polymerisable ester monomer of poly(ethylene glycol).

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In some embodiments of the kit the anti-fouling composition contains a polymeric material containing a positively charged species selected from the group consisting of protonated primary amino, protonated secondary amino, protonated tertiary amino and quaternary ammonium groups, or a negatively charged species selected from the group consisting of carboxylate groups, sulfonate groups, sulfate groups and phosphonate groups.

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In some embodiments of the kit the anti-fouling composition contains a polymeric material containing a positively charged species selected from the group consisting of

protonated primary amino, protonated secondary amino, protonated tertiary amino and quaternary ammonium groups.

5 In other embodiments of the kit the anti-fouling composition contains a polymeric material containing a negatively charged species selected from the group consisting of carboxylate groups, sulfonate groups, sulfate groups and phosphonate groups.

10 In some embodiments of the kit the anti-fouling composition contains a copolymer prepared by polymerization of a polymerisable ester monomer of poly(ethylene glycol) and a sulfonated ethylenically unsaturated monomer. In some specific embodiments the sulfonated ethylenically unsaturated monomer is 4-styrene sulfonate. In some specific embodiments the anti-fouling composition contains a block copolymer of sodium 4-styrenesulfonate and poly(ethylene glycol) methyl ether acrylate.

15 In other embodiments of the kit the anti-fouling composition contains a random copolymer of an acrylate with pendant alkyne groups and a polymerisable ester monomer of poly(ethylene glycol).

20 In other embodiments of the kit the anti-fouling composition contains a random copolymer of an acrylate with pendant azide groups and a polymerisable ester monomer of poly(ethylene glycol).

25 In many of the embodiments of the kit described above the composition (either primer or anti-fouling) contains a copolymer formed by polymerisation of a polymerisable ester monomer and another monomer. In essence a wide range of polymerisable ester monomers may be employed in the present invention although in some embodiments the polymerisable ester monomer is selected from the group consisting of poly(ethylene glycol) acrylates, poly(ethylene glycol) methacrylates, poly(ethylene glycol) methyl ether acrylates and poly(ethylene glycol) methyl ether methacrylates.

30 As stated above the anti-fouling composition can contain a polymeric material having side chains which inhibit biological fouling. In some embodiments the polymeric material has side chains containing one or more ethylene oxide units. In some embodiments each side chain contains between 1 and 1000 ethylene oxide units. In some other embodiments each side chain contains between 1 and 100 ethylene oxide units. In still other embodiments each side chain contains between 1 and 20 ethylene oxide units. In still other embodiments each side chain contains between 3 and 12 ethylene oxide units.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the chemical structures of the block copolymer of PSS and PEG methyl ether acrylate. (PSS-PEG-1: $x=1500$, $y=15$, $n=6$, PSS-PEG-2: $x=330$, $y=50$, $n=6$).

Figure 2 shows the chemical structures of the random copolymer of alkyne- and azide-functional acrylate and PEG methyl ether acrylate. (1) Click-PEG-Alk: alkyne functional acrylate: PEG methyl ether acrylate = 1:9). (2) Click-PEG-Az: azide functional acrylate: PEG methyl ether acrylate = 1:9.

Figure 3 shows the chemical structure of the random copolymer of glycidyl methacrylate and PEG methyl ether methacrylate.

Figure 4 shows the build-up of PSS-PEG 2/PAH and PSS/PAH multilayer films, as measured by UV-Vis spectrophotometer. The polyelectrolyte concentrations were 1 mg mL^{-1} in an aqueous solution also containing 0.5 M NaCl.

Figure 5 shows the incorporation of rhodamine isothiocyanate-labelled PAH into PSS-PEG 2/PAH and PSS/PAH multilayer films, as measured by UV-Vis spectrophotometry. The polyelectrolyte concentrations were 1 mg mL^{-1} in an aqueous solution also containing 0.5 M NaCl.

Figure 6 shows the AFM images of (top) (PSS-PEG 2/PAH)₂₁- and (bottom) (PSS/PAH)₂₁- coated silicon surfaces. The step decrease in thickness on the left section of each image is the result of scalpel blade scratching, from which dry coating thickness is measured.

Figure 7 shows the dissipation (a) and frequency (b) responses recorded over a 5 hour period for the settlement of the diatom *Haslea* sp. upon gold, PSS, PSS-PEG 1 and PSS-PEG 2 using comparable concentrations. Cells were introduced into the QCM chamber at $t=0$.

Figure 8 shows the dissipation (a) and frequency (b) responses recorded over an 18 hour period for the settlement of *Haslea* sp. upon click-PEG surface. The cells were introduced into the QCM chamber at $t=0$.

Figure 9 shows the dissipation (a) and frequency (b) responses recorded over a 5 hour period for the settlement of the diatom *Amphora coffeaeformis* upon gold, PSS, PSS-PEG 1 and PSS-PEG 2 using comparable concentrations. Cells were introduced into the QCM chamber at $t=0$.

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Figure 10 shows the frequency responses for the settlement of the diatom *Haslea sp.* upon (a) native gold and (b) (PSS-PEG 1/PAH)₃PSS-PEG 1 over an 18 h period. Cells were introduced into the QCM chamber at $t=0$. The natural drift of the instrument in sterile seawater has previously been determined to be $f = -7.92\text{Hz}$ over 18h. The frequency response induced by *Haslea sp.* on (PSS-PEG 1/PAH)₃PSS-PEG 1 was less than the natural drift of the instrument, indicating negligible adhesion.

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Figure 11 shows the frequency responses for the settlement of the diatom *Haslea sp.* upon (a) native gold, (b) (PSS-PEG 2/PAH)₁PSS-PEG 2, (c) (PSS-PEG 2/PAH)₂PSS-PEG 2 and (d) (PSS-PEG 2/PAH)₃PSS-PEG 2 over an 18 h period. Cells were introduced into the QCM chamber at $t=0$. The natural drift of the instrument in sterile seawater has previously been determined to be $f = -7.92\text{Hz}$ over 18h. The frequency response induced by *Haslea sp.* on (PSS-PEG 2/PAH)₃PSS-PEG 2 was less than the natural drift of the instrument, indicating negligible adhesion.

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Figure 12 shows a representative frequency response, Δf (Hz), for QCM sensor crystal immersed in sterile diatom growth media (K-medium) over an 18 h period.

Figure 13 shows the frequency responses for the settlement of the diatom *Amphora coffeaeformis* upon (a) native gold and (b) (PSS-PEG 1/PAH)₃PSS-PEG 1 over an 18 h period. Cells were introduced into the QCM chamber at $t=0$. The natural drift of the instrument in sterile seawater has previously been determined to be $f = -7.92\text{Hz}$ over 18h. The frequency response induced by *A. coffeaeformis* on (PSS-PEG 1/PAH)₃PSS-PEG 1 was less than the natural drift of the instrument, indicating negligible adhesion.

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Figure 14 shows the frequency responses for the settlement of the diatom *Amphora coffeaeformis* upon (a) native gold, (b) PEI-PSS-PEG 2, (c) (PSS-PEG 2/PAH)₁PSS-PEG 2, (d) (PSS-PEG 2/PAH)₂PSS-PEG 2 and (e) (PSS-PEG 2/PAH)₃PSS-PEG 2 over an 18 h period. Cells were introduced into the QCM chamber at $t=0$. The natural drift of the instrument in sterile seawater has previously been determined to be $f = -7.92\text{Hz}$ over 18h. The frequency response induced by *A. coffeaeformis* on all surfaces containing PSS-PEG 2 was less than the natural drift of the instrument, indicating negligible adhesion.

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Figure 15 shows the frequency responses for settlement of zoospores of the green alga *Ulva* *sp.* (formerly *Enteromorpha* *sp.*) upon (a) native gold and (b) (PSS-PEG 1/PAH)₃PSS-PEG 1 over a 5h period. Cells were introduced into the QCM chamber at $t=0$. The natural drift of the instrument in sterile seawater has previously been determined to be $f = -4.91$ Hz over 5h. The frequency response induced by *Ulva* *sp.* zoospores on (PSS-PEG 1/PAH)₃PSS-PEG 1 was significantly higher than the natural drift of the instrument.

Figure 16 shows the frequency responses for settlement of zoospores of the green alga *Ulva* *sp.* upon (a) native gold, (b) PEI-PSS-PEG 2, (c) (PSS-PEG 2/PAH)₁PSS-PEG 2, (d) (PSS-PEG 2/PAH)₂PSS-PEG 2 and (e) (PSS-PEG 2/PAH)₃PSS-PEG 2 over a 5h period. Cells were introduced into the QCM chamber at $t=0$. The natural drift of the instrument in sterile seawater has previously been determined to be $f = -4.91$ Hz over 5h. The frequency response induced by *Ulva* *sp.* zoospores on all PSS-PEG 2 test surfaces was less than the natural drift of the instrument, indicating negligible adhesion.

Figure 17 shows the dissipation (a) and frequency (b) responses recorded over a 5 hour period for the settlement of the green seaweed *Ulva* *sp.* zoospores upon gold, PSS, PSS-PEG 1 and PSS-PEG 2 using comparable concentrations. Zoospores were introduced into QCM chamber at $t = 0$.

Figure 18 shows the frequency responses for the settlement of the diatom *Haslea* *sp.* upon (a) native gold and (b) (PVA/PEG_{EPOX})₁ over an 18 h period. Cells were introduced into the QCM chamber at $t=0$. The natural drift of the instrument in sterile seawater has previously been determined to be $f = -7.92$ Hz over 18h. The frequency response induced by *Haslea* *sp.* on (PVA/PEG_{EPOX})₁ was less than the natural drift of the instrument, indicating negligible adhesion.

Figure 19 shows the frequency responses for the settlement of the diatom *Haslea* *sp.* upon (a) native gold and (b) (PVA/PEG_{EPOX})₂ over an 18 h period. Cells were introduced into the QCM chamber at $t=0$. The natural drift of the instrument in sterile seawater has previously been determined to be $f = -7.92$ Hz over 18h. The frequency response induced by *Haslea* *sp.* on (PVA/PEG_{EPOX})₁ was less than the natural drift of the instrument, indicating negligible adhesion.

Figure 20 shows the frequency responses for the settlement of the diatom *Haslea* *sp.* upon (a) native gold and (b) (PVA/PEG_{EPOX})₃ over an 18 h period. Cells were introduced into the

QCM chamber at $t=0$. The natural drift of the instrument in sterile seawater has previously been determined to be $f = -7.92\text{Hz}$ over 18h. The frequency response induced by *Haslea sp.* on $(\text{PVA/PEG}_{\text{EPOX}})_1$ was less than the natural drift of the instrument, indicating negligible adhesion.

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Figure 21 shows the bacterial percentage cover on glass slides coated with $(\text{PSS-PEG } 1/\text{PAH})_3\text{PSS-PEG } 1$, $(\text{PSS-PEG } 2/\text{PAH})_3\text{PSS-PEG } 2$ and the foul-release coating Intersleek 700® following incubation in a 5L laboratory seawater microcosm for 72 h. Acid washed glass slides were used as a control. Data are means $\pm$ 95% CI ($n=3$). Data that are significantly different from the control according to a one-way ANOVA ($P<0.05$) are indicated by an asterisk above the bars.

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Figure 22 shows the bacterial percentage cover on glass slides coated with $(\text{PSS-PEG } 1/\text{PAH})_3\text{PSS-PEG } 1$, $(\text{PSS-PEG } 2/\text{PAH})_3\text{PSS-PEG } 2$ and $(\text{PSS-PEG } 2/\text{PAH})_3$ following immersion at DSTO Research Facility, Williamstown for 72 h in the ocean. Acid washed glass slides were used as a control. Data are means $\pm$ 95% CI ($n=3$). Data that are significantly different from the control according to a one-way ANOVA ($P<0.05$) are indicated by an asterisk above the bars. Percentage cover of fouling on $(\text{PSS-PEG } 1/\text{PAH})_3\text{PSS-PEG } 1$ and $(\text{PSS-PEG } 2/\text{PAH})_3\text{PSS-PEG } 2$ was 0.45 ± 0.11 and 0.08 ± 0.03 respectively.

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Figure 23 shows representative images illustrating the bacterial colonisation upon acid washed glass (a,b), Intersleek 700 (c,d), PEG1 (e,f) & PEG2 (g,h) after 6 days of immersion in the ocean. Control images of areas upon the substrate surface not exposed to shear (a,c,e,g) are compared to areas exposed to shear forces (b,d,f,h).

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Figure 24 shows the mean number of *Haslea sp.* cells adhered to (a) Intersleek 700®, (b) acid washed glass, (c) $(\text{PVA/PEG}_{\text{EPOX}})_1$, (d) $(\text{PVA/PEG}_{\text{EPOX}})_2$ and (e) $(\text{PVA/PEG}_{\text{EPOX}})_3$ following exposure to shear stress of 60 Lmin^{-1} and 120 Lmin^{-1} for 5 min in a fully turbulent flow chamber. Cells were settled for 6h prior to analysis. Data shown are means of three replicate slides for each treatment.

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Figure 25 shows the percentage area cover of *Ulva sp.* zoospores on (a) Intersleek 700® (b) acid washed glass, (c) $(\text{PVA/PEG}_{\text{EPOX}})_1$, (d) $(\text{PVA/PEG}_{\text{EPOX}})_2$ and (e) $(\text{PVA/PEG}_{\text{EPOX}})_3$ following exposure to shear stress of 60 Lmin^{-1} and 120 Lmin^{-1} for 5 min in a fully turbulent flow chamber. Cells were settled for 6h prior to analysis. Data shown are means of three replicate slides for each treatment.

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DETAILED DESCRIPTION OF THE INVENTION

The term "biological fouling" when used herein refers to the fouling of a surface by biological organisms when exposed to an environment capable of hosting such organisms. It includes the direct adsorption of organic fouling molecules onto the surface, attachment of organisms such as bacteria, protists, fungi, tubeworms, sponges, barnacles, mollusks, and other aquatic organisms to the surface, growth of said organisms on the surface, and deposits left by said organisms on the surface.

The method of the present invention may be applied to the protection of the surfaces of a broad range of objects from biological fouling. In essence the methods may be applied to the surface of any object that requires protection from biological fouling. As stated previously the methods of the invention find particular application in the protection of the surfaces of objects in aquatic environments, particularly marine environments.

In some embodiments, the object is exposed to an aquatic environment. In certain embodiments, the object is exposed to marine environments. In certain embodiments, the object is exposed to estuarine environments. In other embodiments, the object is exposed to process water in an industrial environment. In other embodiments, the object is exposed to freshwater environments.

In certain embodiments the object to be subjected to the methods of the present invention is a means of transport used in an aquatic environment. Examples of objects of this type includes a ship, boat, yacht, dinghy, submarine, ferry, speedboat, frigate, aircraft carrier, minesweeper, ice breaker, rowboat, skiff, jet ski, ketch, yawl, sloop, schooner, tanker, barge, or tugboat.

In certain embodiments the object to be subjected to the methods of the invention is a recreational object used in an aquatic environment. Examples of objects of this type include a surfboard, boogie board, windsurfer or waterski.

In certain embodiments the object to be subjected to the methods of the invention is a functional object that is generally exposed to an aquatic environment either permanently or intermittently during use. Examples of objects of this type include a buoy, pier, wharf, jetty, beacon, breakwater, oil rig, fish farm fence, shark net, fishing net, pontoon, crab pot, lobster pot, shark cage or dock.

In certain embodiments of the invention the object to be subjected to the methods of the invention are components of a larger object. In these circumstances the object may be treated whilst part of the larger item or it may be treated as an individual item either by removal from the larger item or before it is added to the larger item. Examples of objects of this type include a keel, fin, mast, sail, rudder, propeller, periscope or cabin.

In certain embodiments of the invention the object to be subjected to the methods of the invention are the glass surfaces of aquaria (domestic and commercial), ornamental rocks and statuary in ponds, underwater viewing vessels such as glass bottomed boats, semi-submersibles.

As can be seen from the above there are a wide range of objects that may be subjected to and benefit from the methods of the present invention. The objects range from such large objects as ships (supertankers and the like) to quite small components such as the propeller of a boat.

The surface of the object to be treated is typically a surface that is either constantly or intermittently in contact with the aquatic environment. This for example in relation to a boat or a ship the surface is typically the hull or a portion thereof. Nevertheless other surfaces such as the deck or inner surface of ballast water tanks of the ship or boat may also benefit from the method of the present invention.

The first step in the method of the invention is the provision of a first functional group on the surface of the object. The nature of this step will vary considerably depending upon the base nature of the surface to be protected. For example in certain circumstances the process of manufacture of the surface in the construction of the object will lead to a surface that inherently has the required first functional groups on the surface. For example the first functional group may be inherent to the surface, such as hydroxyl groups provided by the surface of wood.

In circumstances in which the first functional group is not inherent on the surface it is necessary to introduce a first functional group onto the surface. This may be carried out in a number of ways. For example the first functional group may be introduced by activation of the surface.

Activation of the surface may be carried out in a number of ways known in the art. For example the surface may be activated by direct chemical modification of the surface leading

to a surface with first functional groups thereon. For example, this may be by oxidation with an oxidizing agent, such as an inorganic oxidizing agent or an organic oxidizing agent. Alternatively, the functional group can be provided by exposing the surface to radiation, such as ultraviolet, visible infrared or gamma radiation. In relation to certain circumstances (especially those that contain amine groups on the surface) a functional group may be provided on the surface by acidification of the surface.

In other embodiments the first functional group is provided on the surface by reaction of the surface with a chemical entity to provide a first functional group on the surface. The nature of the chemical entity used in the reaction of the surface will vary based on a number of variables such as the nature of the surface and the chemical moieties inherently present thereon and the desired first functional group to be provided on the surface.

The first functional group may be selected from the group consisting of carboxylate groups, sulfonate groups, sulfate groups, phosphonate groups, protonated primary amino groups, protonated secondary amino groups, protonated tertiary amino groups, quaternary ammonium groups, acyl bromide groups, acyl chloride groups, aldehyde groups, alkene groups, alkyne groups, amide groups, primary, secondary or tertiary amine groups, anhydride groups, azide groups, nitrile groups, carboxylic acid groups, epoxide groups, hydroxyl groups, isocyanate groups, isothiocyanate groups and thiol groups. A skilled worker in the field would be able to determine suitable methodology for the insertion of a functional group of this type.

In some embodiments, the chemical groups extending from the surface are selected from the group consisting of protonated primary amino groups, protonated secondary amino groups, hydroxy groups, alkyne groups and azide groups. In the methods of the present invention the nature of the first functional group will typically be determined based on the nature of the second functional group of the antifouling composition used in the invention as the intention is that the first functional group and the second functional group be complementary such that they will form a bond.

Whilst the first functional group may be provided on the surface using the methods described above the maximum flexibility in selection of the first functional group is provided by applying to the surface a primer composition which includes a material containing the first functional group.

The primer composition

The primer composition may be any suitable primer composition although it typically contains a polymeric material containing the first functional group. A large number of suitable polymeric materials may be used with the identity of the polymeric material to be used in the primer composition being chosen in order to provide the desired first functional group.

One example of a suitable polymeric material for use in the primer composition is a polymeric material having functional groups which are able to undergo reaction with an epoxide group. Suitable polymeric materials include those having hydroxyl groups, amine groups and thiol groups. Such polymer materials may be formed by copolymerization or homopolymerization of an appropriate precursor monomer, or by post synthesis modification of a polymer. Examples of suitable polymeric materials having hydroxyl groups include poly(vinyl alcohol) or poly(vinyl phenol). Examples of polymeric materials having amine groups include polymeric materials such as poly(ethyleneimine), poly(allylamine) poly(N,N-dimethylaminoethyl methacrylate), poly(N,N-dimethylaminoethyl acrylate), or poly(vinylamine). Examples of polymeric materials having thiol groups include poly(methacrylic acid) conjugated with cystamine in the presence of dithiothreitol, or poly(acrylic acid) conjugated with cystamine in the presence of dithiothreitol. Other hydroxy-, amine- and thiol- containing polymers or copolymers suitable for use with the invention would be known to one having skill in the art.

Another example of a suitable polymeric material for use in a primer composition is a polymeric material containing a positively charged species selected from the group consisting of protonated primary amino, protonated secondary amino, protonated tertiary amino and quaternary ammonium groups, or a negatively charged species selected from the group consisting of carboxylate groups, sulfonate groups, sulfate groups and phosphonate groups.

If the first functional group is a positively charged species then it is desirable that the primer composition contains a polymeric material containing a positively charged species selected from the group consisting of protonated primary amino, protonated secondary amino, protonated tertiary amino and quaternary ammonium groups. The first functional group will desirably be a positively charged species when the antifouling composition contains a polymeric material that contains a negatively charged second functional group.

In circumstances where the first functional group is a positively charged species it is desirable that the primer composition contains a polyamine. Any suitable polyamine may be employed however it is found that particularly suitable polyamines include polyamines

selected from the group consisting of poly(ethyleneimine), poly(allylamine hydrochloride) and poly(vinylamine hydrochloride).

If the first functional group is a negatively charged species then it is desirable that the primer composition contains a polymeric material containing a negatively charged species selected from the group consisting of carboxylate groups, sulfonate groups, sulfate groups and phosphonate groups. The first functional group will desirably be a negatively charged species when the antifouling composition contains a polymeric material that contains a positively charged second functional group.

The material in the primer composition containing the first functional groups may also be chosen such that the first functional group is suitable for reaction with the second functional group to form a covalent bond. In some embodiments, the formation of the bond between the chemical group extending from the surface and the chemical moiety on the polymeric material is a so called "click" reaction. In other embodiments, the reaction is a condensation reaction. In still other embodiments, the reaction is a ring opening reaction. In other embodiments, the reaction is a cyclization reaction. In each of these instances the material in the primer composition will be chosen to provide the desired first functional group to achieve the desired reaction

In some specific embodiments, the formation of the bond between the chemical group extending from the surface and the chemical moiety on the polymeric material occurs via the formation of a heterocyclic ring structure. In some embodiments, the heterocyclic ring is formed in the presence of a catalyst. Preferably, the catalyst includes a metal selected from the group consisting of Au, Ag, Hg, Cd, Zr, Ru, Fe, Co, Pt, Pd, Ni, Cu, Rh and W. More preferably, the catalyst includes a metal selected from the group consisting of Ru, Pt, Ni, Cu and Pd. Even more preferably, the catalyst includes Cu(I). Most preferably the heterocyclic ring formation occurs in the absence of a catalyst. The use of elevated temperature or pressure reaction conditions or irradiation (such as by microwaves), may eliminate the need to use a catalyst.

In some embodiments, the bonds formed between the chemical groups extending from the surface and the chemical moieties in the polymeric material result in the formation of a 1,2,3-triazole. In some embodiments, this occurs by the Cu(I) catalysed variant of the Huisgen 1,3-dipolar cycloaddition.

When it is desired that the first functional group form a bond with the second functional group via click chemistry it is desired that the primer composition include a material containing click functional groups. An example of a suitable click chemistry functional group is an alkyne group or an azide group. In some embodiments the primer composition contains
5 a block, gradient, star or random copolymer of an acrylate with pendant azide groups and a polymerisable ester monomer of poly(ethylene glycol), or a block, gradient, star or random copolymer of an acrylate with pendant alkyne groups and a polymerisable ester monomer of poly(ethylene glycol). In some specific embodiments the copolymer is a random copolymer.

10 The selection of the first functional group on the material in the primer composition will depend upon the identity of the second functional group on the polymeric material of the anti-fouling composition. The identity of the first functional group will be chosen to be complementary with the identity of the functional group on the polymeric material of the anti-fouling composition. Thus for example if the first functional group is an alkyne then the
15 second functional group will be an azide. Alternatively if the first functional group is an azide then the second functional group will be an alkyne. A number of complementary click functional group pairings are known in the art and a skilled addressee can easily select a required combination.

20 The primer composition typically utilizes a suitable solvent for the material containing the first functional group. The identity of the solvent will typically be determined based on a number of variables such as cost and compatibility with the material containing the first functional group. Examples of suitable solvents include water, C₁₋₁₀ alcohols, C₁₋₁₀ ketones, C₁₋₁₀ ethers, aromatic solvents, heteroaromatic solvents, C₁₋₁₀ alkanes, N,N-di(C₁₋₁₀)alkyl
25 amides, C₃₋₁₀ cycloalkanes, halogenated solvents, tetrahydrofuran, C₁₋₆ glycol, dimethylsulfoxide and dioxane. In some specific embodiments, the solvent is chosen from the group consisting of water, ethanol, methanol or acetone. In some embodiments the solvent is a mixture of at least two of the solvents listed above. In some specific embodiments the solvent is water.

30 In some embodiments the primer composition includes further additives. In some embodiments the additives are selected from the group consisting of mineral acids, bases and ionic salts. In some embodiments, the additive is sodium chloride. In some other embodiments, the additive is dilute aqueous hydrochloric acid. In still other embodiments the
35 additive is dilute aqueous sodium hydroxide.

The anti-fouling composition

The methods of the invention utilize an anti-fouling composition containing a polymeric material, the polymeric material (i) containing side chains of polymeric moieties which inhibit biological fouling and (ii) containing a second functional group.

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In some embodiments, the polymeric moieties which inhibit biological fouling include vinyl pyrrolidone units or ethylene oxide units. In some embodiments, the polymeric moieties which inhibit biological fouling include ethylene oxide units.

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In some specific embodiments, the methods of the invention utilize an antifouling composition containing a polymeric material, the polymeric material (i) having side chains containing one or more ethylene oxide units and (ii) containing a second functional group.

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As discussed above the identity of the polymeric material used in the anti-fouling composition will be determined based on the identity of the first functional groups on the surface and vice versa. Where the polymeric material of the primer composition includes a hydroxyl- thiol- or amino-containing polymeric material, some suitable polymeric materials for use in the antifouling material will include those having epoxide groups. The epoxide groups may be added to the polymer by co-polymerizing an epoxide containing monomer or by modification of an appropriate precursor polymer with an appropriate reagent post-polymerization. Examples of suitable polymeric materials include those formed by copolymerizing an appropriate epoxide-functional monomer (such as glycidyl methacrylate or glycidyl acrylate) with a polymerizable ester monomer of poly(ethylene glycol), or a polymerizable ester monomer of poly(ethylene glycol) monomethyl ether. One specific polymer is the copolymer of glycidyl acrylate with poly(ethylene glycol) monomethyl ether acrylate. Another specific polymer is the copolymer of glycidyl methacrylate with poly(ethylene glycol) monomethyl ether methacrylate.

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Where the polymeric material in the anti-fouling composition includes an epoxide group, those epoxide groups may be disposed in the polymer backbone, on side chains or on the chain ends of polymeric branches. If disposed on side chains, the epoxide groups may be located in separate branches to the polymeric moieties which inhibit biological fouling, or in the same branches that inhibit biological fouling. If disposed in the sidechains that inhibit biological fouling, the epoxide groups may be located at the end of the side chain closest to the polymeric backbone, or at the end of the side chain furthest from the polymeric backbone. The epoxide groups may be incorporated into the polymer by using an epoxide functional monomer such as glycidyl acrylate or glycidyl methacrylate in the synthesis of the polymer, by

reacting a pre-formed polymer with a reagent suitable for forming epoxide groups on the polymer chain, or by conjugating a preformed polymer with an appropriate reagent having epoxide groups. Some examples of polymeric materials suitable for use in the antifouling composition include copolymers of glycidyl acrylate with poly(ethylene glycol) acrylate, glycidyl acrylate with poly(ethylene glycol) monomethyl ether acrylate, glycidyl methacrylate with poly(ethylene glycol) methacrylate, glycidyl methacrylate with poly(ethylene glycol) monomethyl ether methacrylate, glycidyl methacrylate with poly(ethylene glycol) acrylate, glycidyl methacrylate with poly(ethylene glycol) monomethyl ether acrylate, glycidyl methacrylate with poly(ethylene glycol) acrylate, and glycidyl methacrylate with poly(ethylene glycol) monomethyl ether acrylate.

If the identity of the first functional group is such that the bond between the first functional group and the second functional group is an ionic bond then this will typically occur where the first functional group is a charged species. In these embodiments the anti-fouling composition contains a polymeric material containing a positively charged species selected from the group consisting of protonated primary amino, protonated secondary amino, protonated tertiary amino and quaternary ammonium groups, or a negatively charged species selected from the group consisting of carboxylate groups, sulfonate groups, sulfate groups and phosphonate groups.

In some embodiments the anti-fouling composition contains a polymeric material containing a positively charged species selected from the group consisting of protonated primary amino, protonated secondary amino, protonated tertiary amino and quaternary ammonium groups.

In other embodiments the anti-fouling composition contains a polymeric material containing a negatively charged species selected from the group consisting of carboxylate groups, sulfonate groups, sulfate groups and phosphonate groups.

In certain embodiments of the anti-fouling compositions where it is desirable to have a negatively charged second functional group it is desirable that the copolymer is prepared by polymerization of a polymerisable ester monomer of poly(ethylene glycol) and a sulfonated ethylenically unsaturated monomer. Any suitable sulfonated ethylenically unsaturated monomer may be used although a particularly suitable monomer of this type is 4-styrene sulfonate. In some specific embodiments the anti-fouling composition contains a block copolymer of sodium 4-styrenesulfonate and poly(ethylene glycol) methyl ether acrylate.

The polymeric material having side chains containing polymeric moieties which inhibit biological fouling used in the anti-fouling composition is typically a copolymer prepared from polymerization of a polymerisable ester of poly(ethylene glycol) and another monomer. The polymerisable ester of poly(ethylene glycol) is typically chosen from the group consisting of poly(ethylene glycol) acrylates, poly(ethylene glycol) methacrylates, poly(ethylene glycol) methyl ether acrylates and poly(ethylene glycol) methyl ether methacrylates. In some embodiments, the polymeric material is a block, gradient, star or random copolymer of the polymerisable ester of poly(ethylene glycol), wherein there is at least one other monomer in the copolymer. In some embodiments the side chains containing one or more ethylene oxide units contain between 1 and 1000 ethylene oxide units. In some specific embodiments, the side chains one or more ethylene oxide units contain between 1 and 100 ethylene oxide units. In some specific embodiments the side chains containing one or more ethylene oxide units contain between 1 and 20 ethylene oxide units. In some more specific embodiments, the side chains containing one or more ethylene oxide units contain between 3 and 12 ethylene oxide units. In these circumstances it is typical that the other monomer used in the polymerization process provides the second functional group. As such the identity of the monomer used in the copolymerisation reaction will be selected to provide the desired group.

The second functional group in the polymeric material in the anti-fouling composition which form bonds with the first functional groups on the surface is typically selected from the group consisting of carboxylate groups, sulfonate groups, sulfate groups, phosphonate groups, protonated primary amino groups, protonated secondary amino groups, protonated tertiary amino groups, quaternary ammonium groups, acyl bromide groups, acyl chloride groups, aldehyde groups, alkene groups, alkyne groups, amide groups, primary, secondary or tertiary amine groups, anhydride groups, azide groups, nitrile groups, carboxylic acid groups, epoxide groups, hydroxyl groups, isocyanate groups, isothiocyanate groups and thiol groups. In some specific embodiments, the second functional group in the polymeric material is selected from the group consisting of sulfonate groups, alkyne groups and azide groups. In preparing the anti-fouling compositions of the present invention a skilled worker will readily be able to select suitable monomers to take part in the copolymerisation reaction to deliver the desired functional group.

In some specific embodiments the anti-fouling composition contains a random copolymer of an acrylate with pendant alkyne groups and a polymerisable ester monomer of poly(ethylene glycol). In some other specific embodiments the anti-fouling composition contains a random copolymer of an acrylate with pendant azide groups and a polymerisable ester monomer of poly(ethylene glycol).

In some embodiments the first functional group is provided by a layer of a block, gradient, star or random copolymer of an acrylate with pendant azide groups and poly(ethylene glycol) methyl ether acrylate and the polymeric material is a block, gradient, star or random copolymer of an acrylate with pendant alkyne groups and poly(ethylene glycol) methyl ether acrylate. In other embodiments the first functional group is provided by a layer of a block, gradient, star or random copolymer of an acrylate with pendant alkyne groups and poly(ethylene glycol) methyl ether acrylate, and the polymeric material is a block, gradient, star or random copolymer of an acrylate with pendant azide groups and poly(ethylene glycol) methyl ether acrylate.

In some circumstances the first functional group is a chemical moiety on the surface and the second functional group is a chemical moiety on the polymeric material of the anti-fouling composition and the bond formed between the first functional group and the second functional group is a covalent bond. In these circumstances the chemical moieties in the polymeric material in the anti-fouling composition are typically selected from the group consisting of acyl bromide groups, acyl chloride groups, aldehyde groups, alkene groups, alkyne groups, amide groups, primary, secondary or tertiary amine groups, anhydride groups, azide groups, nitrile groups, diazonium groups, sulfonyl chloride groups, carboxylic acid groups, epoxide groups, aziridine groups, hydroxyl groups, isocyanate groups, isothiocyanate groups and thiol groups.

The covalent bonds formed by the reaction of the first functional group and the second functional group in the polymeric material in the anti-fouling composition are typically selected from the group consisting of amide, ester, urethane, carbamate, urea, allophonate, biuret, isocyanurate, imine, heterocycle, ether, alkyl, cycloalkyl, thiocarbamate, disulfide, sulfide, anhydride, amine, azide and sulfonamide.

The anti-fouling composition typically utilizes a suitable solvent for the polymeric material containing the ethylene oxide moieties. The identity of the solvent will typically be determined based on a number of variables such as cost and compatibility with the polymeric material containing the ethylene oxide moieties. Examples of suitable solvents include water, C₁₋₁₀ alcohols, C₁₋₁₀ ketones, C₁₋₁₀ ethers, aromatic solvents, heteroaromatic solvents, C₁₋₁₀ alkanes, N,N-di(C₁₋₁₀)alkyl amides, C₃₋₁₀ cycloalkanes, halogenated solvents, tetrahydrofuran, C₁₋₆ glycol, dimethylsulfoxide and dioxane. In some specific embodiments, the solvent is chosen from the group consisting of water, ethanol, methanol or acetone. In some

embodiments the solvent is a mixture of at least two of the solvents listed above. In some specific embodiments the solvent is water.

In some embodiments the anti-fouling composition includes further additives. In some
5 embodiments the additives are selected from the group consisting of mineral acids, bases and ionic salts. In some specific embodiments, the additive is sodium chloride. In some other specific embodiments, the additive is dilute aqueous hydrochloric acid. In still other specific
10 embodiments the additive is dilute aqueous sodium hydroxide. In addition the anti-fouling composition may contain additional additives that enhance the surface. Examples of such additives include pigments and the like.

**Formation of Polymeric materials for use in the primer composition
and the anti-fouling composition.**

As discussed above the primer composition and the anti-fouling composition typically
15 include polymeric materials containing suitable functional groups.

In some embodiments, the functional groups in the polymeric material are provided by using monomers which contain the functional group in synthesising the polymeric material. In some embodiments, the monomers containing the functional groups are acrylic, styrenic,
20 allylic, vinylic or methacrylic monomers. In other embodiments, the functional groups are incorporated into the polymeric material after the polymeric material has been prepared from reactive precursor. In some embodiments the monomers containing the functional groups are styrene derivatives containing acyl bromide groups, acyl chloride groups, aldehyde groups, alkene groups, alkyne groups, amide groups, primary, secondary or tertiary amine groups,
25 anhydride groups, azide groups, nitrile groups, diazonium groups, sulfonyl chloride groups, carboxylic acid groups, epoxide groups, aziridine groups, hydroxyl groups, isocyanate groups, isothiocyanate groups and thiol groups, esters of methacrylic acid containing acyl bromide groups, acyl chloride groups, aldehyde groups, alkene groups, alkyne groups, amide groups, primary, secondary or tertiary amine groups, anhydride groups, azide groups, nitrile groups,
30 diazonium groups, sulfonyl chloride groups, carboxylic acid groups, epoxide groups, aziridine groups, hydroxyl groups, isocyanate groups, isothiocyanate groups and thiol groups, esters of acrylic acid containing acyl bromide groups, acyl chloride groups, aldehyde groups, alkene groups, alkyne groups, amide groups, primary, secondary or tertiary amine groups, anhydride groups, azide groups, nitrile groups, diazonium groups, sulfonyl chloride groups, carboxylic
35 acid groups, epoxide groups, aziridine groups, hydroxyl groups, isocyanate groups, isothiocyanate groups or thiol groups.

In some embodiments, the functional groups in the polymeric materials are provided by preparing the polymeric material using the monomers allylamine, sodium 4-styrenesulfonate, sodium acrylate, sodium methacrylate, acrylic acid, methacrylic acid, sodium 4-vinylbenzoate, 4-vinylbenzoic acid, *tert*-butyl acrylate, *tert*-butyl methacrylate, 4-vinylbenzyl-N,N,N-trimethylammonium chloride, acrylonitrile, vinylbenzyl chloride, glycidyl methacrylate, glycidyl acrylate, propargyl acrylate, methacryloyl chloride, acryloyl chloride, methacryloyl bromide, acryloyl bromide, 4-vinylaniline, crotonaldehyde, acrylamide, N,N-dimethylaminoethyl methacrylate, 4-vinylphenol, vinyl acetate, 2-hydroxyethyl methacrylate, 3-hydroxypropyl methacrylate, 2-hydroxyethyl acrylate, 3-hydroxypropyl acrylate, quaternized and unquaternized N,N-diC₁-C₆-alkyl acrylamide, and acrylate and methacrylate variants of all of the above.

Application of the compositions used in the invention

In the methods of the present invention the primer composition and the anti-fouling composition may be applied using any technique known in the art. For example the compositions may be applied by spraying, dipping or painting and the appropriate procedures in order to utilize these techniques would be known to a skilled addressee.

In some embodiments the primer and the antifouling composition are applied more than once to the object to be coated. This may involve repeating the application of the primer – antifouling composition once (i.e. primer – anti-fouling composition – primer – anti-fouling composition). The application of the primer and antifouling composition may also be repeated twice. In some other cases it may be desirable to repeat application of the primer and antifouling composition three times. In still other cases it may be desirable to repeat the application of the primer and antifouling composition four times. In some certain cases it may be desirable to repeat the application of the primer and antifouling composition five times. In principle, the applications may be repeated as many times as desired by the applicator.

If a primer composition is used there may be a lag time between the application of the primer composition and the anti-fouling composition (a cure time). In other embodiments, the anti-fouling composition is applied to the surface immediately after the primer composition. In some embodiments, there is a defined time between application of the primer composition and application of the anti-fouling composition.

In certain embodiments, the primer composition and/or the anti-fouling composition can be applied by exposing the surface to a solution for a period of time. In some embodiments, the period is less than one hour. In other embodiments it is longer than one

hour and less than one day. In still other embodiments it is more than one day and less than one week.

In some embodiments, there is a rinsing step between providing the applying the primer composition and applying the anti-fouling composition. In other embodiments, there is a drying step between applying the primer composition on the surface and applying the anti-fouling composition on the surface. In still other embodiments, there is both a rinsing step and a drying step between applying the primer composition on the surface and applying the anti-fouling composition on the surface. In some embodiments, drying is accomplished by heating. In other embodiments, drying is accomplished by exposing the surface to a stream of gas (such as air or nitrogen). In still other embodiments, the surface is allowed to dry naturally.

The present invention also provides kits containing a primer composition and an anti-fouling composition as described above.

Examples of materials and methods for use with the compositions and methods of the present invention will now be provided. In providing these examples, it is to be understood that the specific nature of the following description is not to limit the generality of the above description.

EXAMPLES

Introduction

Three polymeric materials have been examined: (i) a block copolymer system with domains of sodium 4-styrenesulfonate units and poly(ethylene glycol) methyl ether acrylate units (Fig. 1, hereafter PSS-PEG); (ii) a random copolymer incorporating poly(ethylene glycol) methyl ether acrylate units and acrylate units functionalised with either azide or alkyne groups (Fig. 2, hereafter click-PEG); and (iii) a random copolymer incorporating poly(ethylene glycol) methyl ether methacrylate units and glycidyl methacrylate units (Fig. 3, hereafter PEG_{EPOX}). In the PEG_{EPOX} the glycidyl groups provide epoxide groups.

The PSS-PEG block copolymer system is applied to the surface by first adsorbing a layer of polyamine (poly(ethyleneimine) or poly(allylamine hydrochloride)) to provide positively charged groups on the surface. These positively charged sites facilitate the attachment of the negatively charged sodium sulfonate domains. The process can be repeated a number of times, using the layer-by-layer (LbL) process to alternately layer the PSS-PEG with

poly(allylamine hydrochloride) (PAH). In this case electrostatic interactions promote the assembly.

A first layer of the click-PEG was attached electrostatically to a surface, the surface having been pretreated so as to provide protonated amine groups on the surface. This was achieved by first adsorbing a layer of polyamine (poly(ethyleneimine) or poly(allylamine hydrochloride)) to provide positively charged groups on the surface. After the first attachment, further layers could be attached by exposing the surface alternately to (i) a random copolymer incorporating poly(ethylene glycol) methyl ether acrylate units and acrylate units functionalised with alkyne groups (10% of units having the alkyne, hereafter click-PEG-Alk); and (ii) a random copolymer incorporating poly(ethylene glycol) methyl ether acrylate units and acrylate units functionalised with azide groups (10% of units having the azide, hereafter click-PEG-Az. The attachment of each subsequent layer is achieved by the reaction of the azides and alkynes catalysed by Cu(I), resulting in the formation of 1,2,3-triazoles links.

An alternative system employing the reaction between epoxide groups and hydroxyl groups was also tested. This system is based on the cyclical deposition of epoxide-containing poly(ethylene glycol) (PEG_{EPOX}) and poly(vinyl alcohol) (PVA), and relies on the formation of ether linkages derived from the ring-opening of the epoxide. First, substrates were coated with PEI and a priming bilayer of PSS and PAH. Thereafter, PEG_{EPOX} and PVA were cyclically deposited. The deposition time of each layer is 5 min, with a water rinsing step in between each exposure.

As control experiments, surfaces functionalized with poly(sodium 4-styrene sulfonate) rather than PSS-PEG were used. Control experiments were also undertaken using (i) gold; (ii) glass; and (iii) Intersleek 700 ® by International Paints/Akzo Nobel, a well known, widely available anti-fouling paint.

Materials

High-purity (Milli-Q) water with a resistivity greater than 18 MΩ cm was obtained from an in-line Millipore RiOs/Origin water purification system. Acrylic acid was purified by vacuum distillation and propargyl acrylate was purified by filtration through neutral alumina (70-230 mesh) immediately prior to use. All other chemicals were purchased from Sigma-Aldrich and used without further purification.

Glass slides used as substrates were cleaned with Piranha solution (70/30 v/v% sulfuric acid : hydrogen peroxide). The slides were then sonicated with 50:50 (isopropanol :

water) for 15 min and finally heated to 60°C for 20 min in RCA solution (5:1:1 water : ammonia : hydrogen peroxide). After each step the slides were washed thoroughly with Milli-Q water. Silicon wafers used for both ellipsometry and AFM were prepared using the above procedure without the Piranha treatment. Gold surfaces for IR measurement were cleaned by immersion in Piranha solution twice and then washed thoroughly with Milli-Q water. All substrates were immersed in poly(ethyleneimine) (~25 KDa) with 0.5 M NaCl for 20 minutes before assembly of the click functionalized polymers. The substrates were then washed with Milli-Q water and dried with a stream of nitrogen. pH measurements were taken with a Mettler-Toledo MP220 pH meter, and the pH values were adjusted with 0.1 M HCl and 0.1 M NaOH.

Example 1 – Synthesis of Poly(ethylene glycol)₃ acrylate with azide functionality (click-PEG-Az)

(a) Synthesis of halogen terminated PEG acrylate: 2-[2-(2-chloroethoxy)-ethoxy]ethanol (10 g), triethylamine (10.31 mL) and hydroquinone (0.12 g) were added to dichloromethane (75 mL) and stirred for 10 min. Acryloyl chloride (5.23 mL) in a further 25 mL dichloromethane was then added drop-wise under argon at 0°C. The reaction was left to stir at 0°C for 60 min and then at room temperature overnight. The reaction was purified by washing with 100 mL water (twice), 0.5 M HCl, 100 mL water (twice), 0.5 M NaOH, brine and then dried with magnesium sulfate (MgSO₄). The crude product was purified by rotary evaporation producing 9.3g of pale yellow liquid.

(b) Synthesis of dodecyl 1-phenylethyl carbonotrithioate: Dodecane thiol (4.8 g), carbon disulphide (3.6 g), triethylamine (4.8 g), and dichloromethane (15 mL) were added to a round-bottom flask and stirred overnight. 1-bromoethyl benzene (3.6 g) in a further 10 mL dichloromethane was added and then the reaction was stirred again overnight. The purity of the reaction was confirmed using TLC (diethyl ether : hexane (3:1)). The product was washed several times with water, brine and then dried over magnesium sulfate. The product was rotary evaporated to produce 6.21 g (83.8% conversion) of yellow solid material. ¹H NMR (CDCl₃): 0.85 (t, CH₂CH₃), 1.23 (s, CH₂CH₂), 1.36 (m, CH₂CH₃), 1.65 (m, CH₂CH₂S), 1.72 (d, CH₃CH), 3.32 (m, CH₂S), 5.30 (CH₃CH), 7.29 (m, benzylic CH).

(c) Polymerization of poly(ethylene glycol)₃ acrylate with azide functionality (click-PEG-Az): Poly(ethylene glycol acrylate)₃ with azide functionality (click-PEG-Az) was synthesized with the following procedure: initial reactants were mixed at approximately a 350:50:1 molar ratio of methoxy terminated poly(ethylene glycol)₃ acrylate (0.854 g), chlorine terminated poly(ethylene glycol)₃ acrylate (0.158 g), and RAFT agent (dodecyl 1-phenylethyl

carbonotrithioate (0.0038 g). 10 wt % Azobisisobutyronitrile (0.2 mg) relative to the RAFT agent was also added along with 3ml dioxane. The solution was purged using three freeze thaw cycles on a Schlenk line and then polymerized at 60°C in a constant temperature oil bath (23 h). The product was dialyzed for 48 h to remove excess monomer. The polymer was then stirred for several days with sodium azide at 60°C (1 g). The final product was then dialyzed again for 48 h and freeze dried.

Example 2 - Synthesis of Poly(ethylene glycol)₃ acrylate with alkyne functionality (click-PEG-Alk)

Poly(ethylene glycol)₃ acrylate with alkyne functionality (click-PEG-Alk) was synthesized using a similar procedure with a molar ratio of approximately 350:50:1 methoxy terminated poly(ethylene glycol)₃ acrylate (1.712 g), acrylic acid (1.01 g) and the above RAFT agent (7.5 mg). 10 wt % Azobisisobutyronitrile (0.2 mg) relative to the RAFT agent was also added along with 3ml dioxane. The solution was purged using three freeze thaw cycles on a Schlenk line and then polymerized at 60°C in a constant temperature oil bath (36 h). The polymer was stirred overnight with propargyl amine (0.010 g) in the presence of 1-[3'-(dimethylamino)propyl]-3-ethylcarbodiimide (0.150 g). The product was dialyzed for 7 days and then freeze dried.

Example 3 - Synthesis of Poly(ethylene glycol)₃ methacrylate with epoxide functionality (PEG_{EPOX})

Poly(ethylene glycol) methacrylate (M_n 526g mol⁻¹) (0.80g), glycidyl methacrylate (55.6mg), a trithiocarbonate transfer agent (4-cyano-4-(dodecylsulfanylthiocarbonyl)sulfanylpentanoic acid) (6.2mg) and AIBN (0.3mg) were added to a schlenk flask. Dioxane (3 mL) was then added and the flask degassed by 4 freeze-thaw cycles prior to the reaction. The polymerization was conducted by immersing the flask in a 60 degree oil bath for 36 hours. The polymer was precipitated twice into diethyl ether. The M_n of the final (waxy) polymer was determined to be approximately 21,000 g mol⁻¹ (by ¹H NMR).

Example 4 – Synthesis of Poly(4-styrenesulfonic acid-block-poly(ethylene glycol) methyl ether methacrylate) (PSS-PEG)

The monomer sodium 4-styrenesulfonate (6g, 0.029 moles) was dissolved in 20 mL distilled water. 3-Benzyl(sulfanylthiocarbonyl)sulfanylpentanoic acid (0.1387 g, 0.0005 moles) and initiator, 4,4'-azobis-4-cyanopentanoic acid (ACPA) (28.6mg, 0.0001 moles) were dissolved in 10 mL ethanol. Two solutions were mixed together and degassed on Schlenk line by three cycles of freeze-pump-thaw, after which they were blanketed with nitrogen. The polymerization was initiated by immersing the glass vial in an oil bath set at 70°C, and the

polymerization allowed to proceed for 16 h. The polymer (PSS-RAFT) was purified by precipitating into acetone, filtering, and drying in a fumehood overnight. Close to 100% conversion was achieved. The M_n determined by NMR was 14,600 g mol⁻¹, which is close the calculated M_n of 12,400 g mol⁻¹. The M_n determined by aqueous GPC was 52,400 g mol⁻¹ with PDI = 1.2.

Block polymer PSSPEG was synthesized by chain extending the above PSS-RAFT. A water-soluble thermal initiator 4,4'-azobis-4-cyanopentanoic acid (ACPA) was used for polymerization, which was conducted in water and at 70°C. Specifically, the monomer polyethylene glycol methyl ether acrylate, PEGMEA (2.2704g, 0.005 moles), macroRAFT agent sodium polystyrene sulphonate, PSS-RAFT (1.24g, 0.0001 moles), and initiator, ACPA (5.6mg, 0.00002 moles) were weighed into a glass vial. Distilled water (13 mL) was added to dissolve all components. The mixture was then degassed on a Schlenk line and blanketed with nitrogen after three degassing cycles. The polymerization was started by immersing the glass vial in an oil bath set at 70°C for 1 hour. The polymer was purified by dialysis followed by freeze drying. 86.7% conversion was determined by ¹H NMR (D₂O as solvent). The NMR determined M_n of the second block (PEMEA) was estimated to be 25,600 g mol⁻¹, which is close the calculated M_n 19,680 g mol⁻¹, although the M_n determined by aqueous GPC was 70,340 g mol⁻¹ and PDI = 1.13.

Example 5 – Preparation of PSS-PEG-containing Coatings

Substrates (glass slides, quartz slides and gold QCM electrodes) were coated with PSS-PEG films by an alternate adsorption procedure. In the case of both glass slides and gold QCM electrodes, the substrate was initially exposed to an aqueous solution of polyethyleneimine for 5 minutes (1 mg mL⁻¹, also containing 0.5 M NaCl), and was then rinsed with water. The substrate was then exposed to an aqueous solution of PSS-PEG (1 mg mL⁻¹, also containing 0.5 M NaCl) for 5 minutes, and was then rinsed again in water. Thereafter the film was alternately exposed to solutions of poly(allylamine hydrochloride) (PAH, 1 mg mL⁻¹, also containing 0.5 M NaCl), and PSS-PEG (1 mg mL⁻¹, also containing 0.5 M NaCl), each for five minutes and with a water rinsing step in between each exposure. After depositing the desired number of layers of PSS-PEG, the substrate was ready for testing. Control surfaces were prepared by following the same procedure, but substituting PSS (M_n = 70 000 g mol⁻¹) for PSS-PEG.

Example 6 – Preparation of Click-PEG-containing Coatings

The following stock solutions were made: (a) PEG-Az (0.83 mg mL⁻¹) (b) PEG-Alk (0.83 mg mL⁻¹), (c) copper sulfate (1.8 mg mL⁻¹) and (d) sodium ascorbate (4.4 mg mL⁻¹). The

final polymer solutions for adsorption were made up in a constant volume ratio of 3(a or b):1(c):1(d). To prevent oxidation of the copper, new copper stock solutions were prepared after deposition of each PEG-Az/PEG-Alk bilayer.

5 The slides were prepared by depositing a layer of PEI onto the glass slide/QCM crystal from a 1 mg mL^{-1} solution containing 0.5 M NaCl . The slides were rinsed 3 times in water after each polymer deposition. A layer of PAA-Az (1 mg mL^{-1}) was electrostatically deposited onto the PEI surface to facilitate covalent attachment of the subsequent PEG-Alk layer. The “click” PEG layers were assembled by alternately coating the surface with PEG-Alk or PEG-Az from the solution containing 0.5 mg mL^{-1} polymer, 0.36 mg mL^{-1} copper sulfate and 0.88 mg mL^{-1} sodium ascorbate.

Example 7 – Preparation of PEG_{EPOX}-containing Coatings

15 An alternative system employing the reaction between epoxide groups and hydroxyl groups was also tested. This system is based on the cyclical deposition of epoxide-containing poly(ethylene glycol) (PEG_{EPOX}) and poly(vinyl alcohol) (PVA). First, substrates were coated with PEI (1 mg mL^{-1} in 0.5 M NaCl), followed by a priming bilayer of PSS and PAH (both 1 mg mL^{-1} in 0 M NaCl). Thereafter, PEG_{EPOX} and PVA were cyclically deposited. The deposition time of each layer is 5 min, with a water rinsing step in between each exposure. After 20 deposition of up to 5 PEG_{EPOX}/PVA layers, the substrates were ready for testing.

Example 8 - Characterization of PSS-PEG2/PAH films

25 The characterization of PSS-PEG 2/PAH films was performed with UV-Vis spectrophotometry, atomic force microscopy and dual polarization interferometry.

30 **(a) UV-Vis Spectrophotometry:** UV-visible spectra were collected using an Agilent 8453 single beam UV-Vis spectrophotometer. A PEI-coated quartz slide blank was taken before each measurement. PSS has an absorption peak at 227 nm, while FITC-PAH has an adsorption peak at 565 nm. Fig. 4 and Fig. 5 revealed that the PSS-PEG 2/PAH multilayer film assembles linearly and follow a build up regime that is very similar to that of the conventional PSS/PAH system.

35 **(b) Atomic Force Microscopy (AFM):** The thickness and morphology of PSS-PEG/PAH- and PSS/PAH-coated silicon wafers were examined with an MFP-3D Asylum Research instrument operated in AC mode. The air-dried samples were imaged in air with BS-Tap300 (Bulgaria) cantilevers. To measure the film thickness, a scalpel blade was used to scratch the films in several areas, and images were taken at several points on the edge of

each scratch (Fig. 6). The distance between two peaks in the height distribution analysis was determined as the film thickness.

(c) **Dual Polarization Interferometry (DPI):** Measurements were carried out on an Analight Bio200 dual polarization interferometer (Farfield Scientific Ltd., Cheshire, U.K.). A continuous flow of deionised water with an adjustable pump rate was introduced into the instrument using a dual syringe pump (Harvard Apparatus PHD 2000, Holliston, MA). The DPI chip was illuminated using light from a helium neon laser (632.8 nm), which is split equally into two beams and transverses both the reference and the sensing waveguides before exiting the structure to form Young's interference fringes in the far-field. The polarization state of the light beam was switched between the transverse magnetic (TM) and transverse electric (TE) modes using a ferroelectric liquid crystal half-wave plate at typical frequencies of 50 Hz by the digital signal processing unit. Samples were injected via a standard HPLC injection port into a sample loop, which held a maximum volume of 200 μL . The amount and rate at which the PSS-PEG 2, PSS and PAH solutions (1 mg mL^{-1} in 0.5 M NaCl) was injected over the chip was controlled using the Analight Bio200 6 $\times$ 6 Inject software. The sample temperature within the instrument was regulated using a precision two-stage temperature Peltier to within 2 mK. Changes in the fringe spatial position (phase change) for both the TM and TE signals were monitored over time and converted to multilayer film thickness and density by the instrument's software.

Table 1: Characterisation of PSSPEG/PAH and PSS/PAH multilayers

	PSS-PEG 2/PAH	PSS/PAH
Dry Thickness (per bilayer) ^a	5 nm	4 nm
Wet Thickness (per bilayer) ^b	16 nm	6 nm
Density ^b	0.31 g cm^{-3}	0.86 g cm^{-3}
Mass Adsorbed ^b	17 ng mm^{-2}	15 ng mm^{-2}

^aDetermined using atomic force microscopy

^bDetermined using dual polarization interferometry from 3.5 bilayer assembly

AFM and DPI data are summarized in Table 1. First, both the PSS-PEG 2/PAH and the PSS/PAH systems contain roughly the same amount of material (within experimental errors). However, the PSS-PEG 2/PAH multilayer film is thicker when measured under dry conditions (AFM). This indicates that polymeric constituents in PSS-PEG 2/PAH film adopts a more coiled and 'fluffy' conformation. When measured under wet conditions (DPI), the

PSS-PEG 2/PAH film is dramatically thicker than the PSS/PAH multilayer film. The difference accentuates the hydration effect of PEG block in the PSS-PEG 2/PAH system.

Example 9 - Quartz Crystal Microbalance Assays of Test Surfaces

Studies on diatom adhesion were conducted using a quartz crystal microbalance (QCM) (Q-Sense QCM-D300 with a Q-Sense Axial Flow Cell (QAFC301) (Q-Sense AB, Västra Frölunda, Sweden). The system was initially equilibrated with deionized water at $23.35 \pm 0.025^\circ\text{C}$. Once all resonant overtone parameters were within the range stipulated by the manufacturer, the system was pre-equilibrated with sterile, enriched seawater K-medium used to culture the cells (Keller, M.D., Selvin, R.C., Claus, W. and Guillard, R.R.L. 1987. "Media for the culture of oceanic ultraphytoplankton" J. Phycol. 23: 633-638), and the f & D were re-acquired in the experimental K-medium. The system was then allowed to stabilize prior to the introduction of cells. K-medium with cells was briefly injected into the instrument flow cell 'loop' to allow for temperature stabilization, and thereafter approx. 0.4 ml of k-medium with suspended cells was introduced to the measurement chamber. To ensure that "comparable" cell concentrations of diatoms and/or zoospores were injected into the QCM, replicate counts using a standard haemocytometer technique were used to standardize cell numbers. Data collected from the 3rd overtone was used for data analysis in all experiments. It has previously been determined that f and D parameter responses upon the injection of diatom cells into the QCM chamber are induced by the interaction between the diatomic adhesives and the substrate surface (For details refer to Molino et al 2006, Utilizing the QCM-D to characterize the adhesive mucilage secreted by two marine diatom species in-situ and in real-time, *Biomacromolecules*, 7[11] 3276-82).

(a) Diatom (*Haslea* sp. and *Amphora coffeaeformis*) settlement experiments on PSS-PEG2/PAH and PSS-PEG1/PAH test surfaces: QCM experiments utilising comparable cell concentrations of the diatom species *Haslea* sp. (Fig. 7 and Fig. 8) and *Amphora coffeaeformis* (Fig. 9) revealed remarkably similar trends for cells settled upon each of the test surfaces. *Haslea* sp. induced negligible parameter responses when settled upon PSS-PEG 1 & PSS-PEG 2, producing f shifts of -5 Hz and -1 Hz, and D shifts of 2 and 3, respectively. There was also negligible impact noticeable on the click-PEG surface (Fig. 8) While cells settled upon PSS also induced minimal f (-3 Hz) and D (5) shifts, those settled upon the native gold sensor surface produced significantly larger shifts. During the first hour post injection into the QCM chamber, *Haslea* sp. induced a Δf of 100 Hz, coupled to a D shift of 52, and thereafter the dissipation decreased slightly for the remainder of the experiment (to 47 by 5 hrs), while f remained stable at ~100 Hz. As has been previously investigated (Molino et al. *Biomacromolecules* 2006, 7, 3276 – 3282), f and D parameter responses upon

the injection of diatom cells into the QCM chamber are induced by the interaction between the diatom adhesives and the substrate surface. The lack of f and D responses produced from comparable number of cells settled upon the PSS-PEG 1, PSS-PEG 2, click-PEG and the PSS surfaces, compared to that for gold, reveals a distinct reduction, or absence, of any interaction between the cellular adhesives and the substrate surface, therefore revealing a lack of any appreciable adhesion between the cells and the surface.

(b) *Haslea sp.* settlement experiments on PSS-PEG 2/PAH test surfaces: The adhesion of *Haslea sp.* to the unmodified control surface (i.e. gold) induced a strong frequency response, concluding at - 56.51Hz at 18 h (see Fig. 5). This is a significant deviation from the natural drift of the instrument, indicating strong adhesive interaction between cells and the sensor surface. The settlement of *Haslea sp.* on (PSS-PEG 1/PAH)₃PSS-PEG 1 and (PSS-PEG 2/PAH)₃PSS-PEG 2 induced maximum frequency responses of - 6.99 Hz and -5.91 Hz respectively (Fig. 10; Fig. 11d. These are less than the natural drift of the instrument (-7.92 Hz at 18 h; Fig. 12), indicating negligible adhesion. Importantly, the adhesion strength of *Haslea sp.* to films of (PSS-PEG 2/PAH)_xPSS-PEG 2 was found to increase markedly as the number of constituent bilayers decreased (Fig. 11 a-c).

(c) *Amphora coffeaeformis* settlement experiments on PSS-PEG 2/PAH test surfaces: The adhesion of *Amphora coffeaeformis* to the unmodified control sensor (i.e. native gold) produced a frequency shift of - 39.93 Hz (see Fig. 13). This is a significant deviation from the natural drift of the instrument, indicating strong adhesive interaction between cells and the sensor surface. In contrast, the PSS-PEG test films demonstrated significant antifouling potential against *A. coffeaeformis*. The settlement of cells on sensors modified with (PSS-PEG 1/PAH)₃PSS-PEG 1 produced a frequency response of -5.42Hz at 18 h (Fig. 13). This parameter response is less than the natural drift of the instrument with sterile K-medium (-7.92 Hz $\pm$ 1.46 at 18 h; Fig. 12, indicating that negligible cellular adhesion occurred. Similarly, the settlement of *A. coffeaeformis* on all test films containing PSS-PEG 2 was found to induce a parameter response no greater than that of the natural drift of the instrument with sterile K-medium (Fig. 14). It is interesting to note that the number of constituent PSS-PEG 2 layers in the test film does not affect its anti-fouling efficacy against *A. coffeaeformis*, in contrast to the results discussed above for *Haslea sp.*

(d) *Ulva sp.* (formerly *Enteromorpha*) settlement experiments on PSS-PEG 2/PAH test surfaces: The adhesion of *Ulva sp.* zoospores to the unmodified control surface (i.e. gold) induced a frequency shift of -32.62 Hz at 5 h (see Fig. 15). This is a significant

deviation from the natural drift of the instrument (-4.91 Hz at 5 h), indicating strong adhesive interaction with the sensor surface. On all PSS-PEG 2 test films, the settlement of *Ulva sp.* zoospores produced a frequency response that was less than the baseline drift of the instrument, indicating negligible adhesion (Fig. 16b-e). Interestingly, settlement of zoospores on (PSS-PEG 1/PAH)₃PSS-PEG 1 induced a significantly larger frequency shift than that recorded on the gold control (-109.05 Hz at 5 h , Fig. 15), indicating particularly strong adhesion.

(e) *Ulva sp.* settlement experiments on PSS-PEG 1/PAH and PSS-PEG 2/PAH

test surfaces: The QCM was utilised to monitor the settlement and subsequent interaction of zoospores of the well known fouling, green seaweed *Ulva sp.* to the test surfaces. Fig. 17 illustrates the frequency (f) and dissipation (D) responses recorded over a 5 hr period upon the injection of zoospores of *Ulva sp.* onto either a native cleaned gold sensor surface, or surface modified by the adsorbing of either PSS, PSS-PEG 1 or PSS-PEG 2. Zoospores settled upon our "control" gold surface induced relatively small f and D responses (Δf of -21 Hz and ΔD of 9), when compared to zoospores settled upon PSS-PEG 1, which induced frequency and dissipation responses of -120 Hz and 27 . Interestingly, while PSS-PEG 1 generated large parameter shifts, PSS-PEG 2 produced only negligible frequency (-3 Hz) and dissipation (2) shifts over the 5 hr study. Similarly, the PSS surface induced a minor frequency shift of -5 Hz and dissipation shift of 2 . The parameter shifts are significant for several reasons. Upon adhesion to a surface, zoospores secrete an adhesive pad which provides permanent adhesion to a surface. Previous to this, the cells are actively moving throughout the chamber, searching for a suitable surface on which to adhere. Therefore, if zoospores actively bind to the substrate surface upon the sensor, significant parameter shifts would be expected as illustrated for the PSS-PEG 1 surface, and to a lesser degree the native gold surface. However, if there is a minimal response in the f and D parameters, then there is no adhesive interaction between the zoospores and the substrate. For zoospores of *Ulva sp.*, this means that either; (i) the zoospores are actively attempting to adhere to the substrate surface, but are incapable of doing so, or (ii) the zoospores do not recognise the presenting surface as a solid surface, or suitable surface, and therefore do not interact with the substrate with the intent to adhere.

(f) *Haslea sp.* settlement experiments on PVA/PEG_{EPOX} test surfaces: The settlement of *Haslea sp.* on all (PVA/PEG_{EPOX})_x test films induced frequency responses of no greater magnitude than the natural drift of the instrument, indicating negligible adhesion (Fig. 18-20). This result indicates that the number of constituent PVA/PEG bilayers does not affect

the anti-fouling properties of (PVA/PEG_{EPOX})_x test films against *Haslea sp.* Studies with *A. coffeaeformis* give similar results (data not shown).

Example 10 - Microscope observations of diatom settlement

5 Microscope slides were imaged using a Panasonic digital 3CCD camera set upon a Leica inverted DMIL microscope, and time lapse video was recorded using a Pioneer re-writable video disk recorder (VDR – V1000P). Time Lapse video recordings were taken at randomly chosen locations upon the sensor surface, for a period of five minutes at each sample location. Adhesion of the cells was ascertained by imaging before and after mild
10 agitation in seawater K-medium. The flow chamber (discussed below) was not required to remove cells, just mild agitation in a beaker.

Diatom cells inoculated onto the gold quickly settle and move over the surface, secreting adhesive mucilage and generating a response by the QCM crystal. On the PSS
15 surface, diatoms likewise settle and instantly start moving over the surface. However, when subjected to flow, diatoms are shown to adhere strongly to gold, but weakly to the PSS. The fact that cells move quickly on the PSS surface indicates that there is enough adhesion to at least generate sufficient traction for movement. Diatoms do not show normal settlement behaviour when placed over the PSS-PEG 1 and PSS-PEG 2 surfaces discussed above.
20 Cells were not seen to adhere to the surface even after several hours, and instead tend to adhere to one another to form clumps of cells that appear to accumulate just off the surface. After 20 hours, some movement was observed on the PSS-PEG 1 surface, but none on the PSS-PEG 2 surface. The click-PEG surface gave the same results as the PSS-PEG 2 surface. In summary, the PSS-PEG surfaces and the click-PEG surface are not recognized
25 by the organisms as surfaces, even after several hours. Eventually, some contact is made with the PSS-PEG 1 surface, and this does allow some movement. With the exception of the gold surface, diatoms are easily removed from the other surfaces by gentle agitation and even mild shear from the flow chamber is not required.

30 As noted previously, the trends for the parameter shifts observed for *A. coffeaeformis* upon each of the test surfaces (Fig. 9) was comparable to that seen for *Haslea sp.* Cells settled upon the PSS-PEG 1 and PSS-PEG 2 surfaces induced negligible *f* and *D* responses over the 5 hr time course, producing *f* shifts of -6 Hz and -2 Hz, and *D* shifts of 3 and -3, respectively. Cells settled upon the gold surface however generated far greater parameter
35 shifts, reaching *f* values of -35 Hz and *D* values of 24 after 5 hrs. Therefore as already noted, the lack of parameter responses recorded for cells settled upon the PEG surfaces is indicative of a failure for the cell to significantly interact and adhere to the substrate surface.

Example 11 - Microscopic observation of *Ulva sp.* zoospore settlement on PSS-PEG 2/PAH test surfaces

Microscope slides were placed in a plastic quadriperm dish, which was subsequently
5 filled with sterile seawater. Seawater with zoospores was then injected above slides and the
zoospores observed over time using a Nikon inverted microscope at low and medium
magnification. A haemocytometer was used to ensure that the cell concentration in
suspension was consistent for all experiments.

10 High concentrations of zoospores inoculated above the surfaces discussed above (at
the same zoospore concentration and from the same sample) show that zoospores can
detect a favourable surface relatively quickly. On gold and (PSS-PEG 1/PAH)₃PSS-PEG 1
surfaces, few swimming zoospores are present in medium after 2 hours, with most settled.
However, on (PSS-PEG 2/PAH)_xPSS-PEG 2, large numbers of swimming zoospores are still
15 unsettled after 4 hours. Eventually, zoospores either settle or die. If another surface is
available (i.e., glass or plastic petrie dish) zoospores appear to preferentially settle on it.

Example 12 - Assays of bacterial adhesion to PSS-PEG 2/PAH test surfaces

The settlement density of a natural population of marine bacteria onto PSS-PEG
20 2/PAH test surfaces was investigated in both field and laboratory based assays.

(a) Data collection: Slides were transferred from field containers to a sample dish
containing sterile seawater. It was ensured that slides remained hydrated at all times. Slides
were gently rinsed to remove any non-adhered cells and then carefully lifted to the surface of
25 the sample dish. A 22 mm x 32 mm cover slip (HD Scientific) was placed on the water
surface just above the slide, and the slide with cover slip removed from the sample dish,
therefore allowing the area under the cover slip to remain hydrated at all times.

The slide surface with adhered organisms was stained and fixed by drawing a solution
30 of 20 mg ml⁻¹ Hoechst 33342 (trihydrochloride) (Molecular Probes: H1399) fluorescent stain
in 2.5% glutaraldehyde in sterile filtered seawater under the coverslip and the coverslip
sealed with Valap (Vaseline, Paraffin and lanolin in a 1:1:1 ratio). The outer edge of the Valap
seal was subsequently coated with a thin layer of nail varnish to enhance the seal.

35 Images of the spatial characteristics of the adhered organisms were obtained using a
Leica DC 300F digital camera attached to an Olympus BH-2 epifluorescence microscope with
a mercury bulb light source. Twenty images at haphazard locations on the slide were taken

using a 40x Olympus UVFL 40 (1.30 oil: 150/1.17) objective with an ultra-violet excitation filter set (334–365 nm) to visualise the adhered bacterial population through the excitation of the Hoechst 33342 nucleic acid stain. This stain allows for the visualisation of the actual bacterial cell, although the surrounding mucilaginous capsule and extracellular polymeric substances (EPS) are not stained. Microscopic images were always taken with the focal plane at the substratum surface.

Images were converted to an indexed colour mode using Adobe Photoshop 6.0.1 graphics editor software. Fluorescent pixels in each image, indicating bacteria, were counted and from this an estimation of percent area cover of bacteria was determined. The mean was taken and an overall percentage area cover was determined for each of the test surfaces. Data was transferred to Minitab 15 statistical analysis software. Data were subjected to one-way ANOVAs to determine differences between test surfaces and the control. Data are reported here as mean $\pm$ 95% CI for the mean. (For detail, see Molino et al. 2009, Development of the primary bacterial microfouling layer on antifouling and fouling release coatings in temperate and tropical environments in Eastern Australia, Biofouling, 1-14).

(b) Laboratory mesocosm study: Seawater was collected from Williamstown, Victoria, on the day of the trial and transferred to a 6 L plastic container in the laboratory to form an artificial mesocosm. The mesocosm was kept at 16°C under Sylvania 15W Cool White fluorescent lamps and a magnetic stirrer was used to maintain constant fluid circulation. Test slides were placed in a slide holder and fully submerged in the seawater for a 72 h incubation period. Three replicate slides were used for each test surface. Fouling on experimental slides was observed to consist entirely of marine bacteria (i.e. no diatoms or other protists). Bacterial coverage of the unmodified control surface (i.e. acid-washed glass) was $4.94 \pm 0.61\%$ (Fig. 21). Both PEGylated films exhibited significantly lower bacterial settlement than the glass control ($P < 0.05$; Fig. 21). Of these, slides modified with (PSS-PEG 2/PAH)₃PSS-PEG 2 were fouled least ($0.47 \pm 0.09\%$; Fig. 21). In contrast, bacterial settlement on the Intersleek 700® foul-release coating was over 30% (Fig. 21).

(c) Field study: Strips of overlapping clear packing tape were placed on 20cm x 34cm fiberglass “fly screening”. A thin layer of Selleys® Wet Area Silicone Sealant (white) was applied thinly on top of the tape. Three test slides of a single treatment were placed in a column on top of the sealant. An additional three slides were placed at either end of the column to minimise artifacts caused by edge effects. It was ensured that there were no gaps between adjoining slides and panels were allowed to dry for 12 h. The fly screen was subsequently wrapped around a 7.5cm x 31cm acrylic panel and secured with nylon cable

ties. Panels were transported to the DSTO Maritime Testing Facility at Tenix, Williamstown, Victoria, where they were bolted to vertically suspended aluminium frames situated on a floating raft. All panels were submerged approximately 1.5 ft below water level for a 72h incubation period. Thereafter, each panel was transferred to a plastic container of seawater and returned to the laboratory. In general, results showed a similar trend to that reported for the laboratory study. Fouling on experimental slides was composed entirely of marine bacteria. On the unmodified control surface, bacterial coverage was $2.49 \pm 0.87\%$ (Fig. 22). Both PEGylated PEM films showed significantly lower fouling than the control ($P < 0.05$). As under laboratory conditions, slides modified with (PSS-PEG 2/PAH)₃PSS-PEG 2 showed least fouling (less than 0.1 %; Fig. 22).

In both laboratory and field-based assays, a considerable bacterial film was observed on all slides modified with PEGylated PEM films prior to rinsing. However, the strength of adhesion of the settled cells was so low that even the minimal shear forces created by gentle movement of the slide in seawater were sufficient to remove the film *en masse*.

Example 13 - Settlement of bacteria from seawater onto experimental surfaces and controls

Here the colonisation of PEG-modified surfaces and controls by marine bacteria was investigated. Bacterial colonisation of four surfaces was compared; PSS-PEG 1, PSS-PEG 2, Intersleek 700[®] marine coating, and acid washed glass (a control surface). Replicate glass microscope slides modified with each surface were randomly arranged in a slide holder and incubated in natural seawater for 6 days. The seawater was constantly stirred by a magnetic stirrer and kept at ~16 degrees Celsius at a 10:14 hr light/dark cycle growth chamber. At 6 days, the slides were removed from the slide holder (being kept hydrated at all times), and placed in a smaller dish where half of each slide was subjected to a hydrodynamic 'shear force' by forcing water across the slide surface at velocity using a plastic pipette. Fig. 23 contains representative images of the substrate surfaces that were either subjected to a simple shear stress test (b,d,f,h) or not subjected to shear stress (a,c,e,g).

The bacterial colonisation of the Intersleek[®] substrate surface measured here after 6 days is roughly comparable to a far more detailed and thorough field trial conducted by Paul Molino (PhD student: unpublished data), and therefore this laboratory study is considered to be a reliable example of biofilm formation in the field. It was found that there was a stark contrast in both the bacterial percentage area cover after 6 days between the surfaces, as well as the percentage of bacterial removal after slight shear forces were applied. The acid washed glass surface showed no significant bacterial removal, with ~14% percentage

bacterial cover on areas both exposed to slight shear, as well as those left untreated (Fig. 23a,b). While bacterial colonisation upon Intersleek was far greater, ~40% on untreated areas (Fig. 23c), there was no detectable bacterial removal when this surface was subjected to slight shear, with areas exposed to shear displaying approx. the same bacterial cover (~40%). The PEG-modified surfaces revealed a bacterial percentage cover of ~13% for PSS-PEG 1 (Fig. 23e) and 10% for PSS-PEG 2 (Fig. 23g) for untreated substrate areas. While this is roughly comparable to glass, these two surfaces were found to exhibit significant bacterial removal when exposed to slight shear, with both PSS-PEG 1 and PSS-PEG 2 exhibiting <1% bacterial cover (Fig. 23f,g). However, between these two surfaces, the PSS-PEG 2 surface had far fewer bacteria attached than the PSS-PEG 1 following slight shear. The images are representative, and were chosen to show that occasionally bacteria do attach. Many views on the PSS-PEG 2 surface were devoid of bacteria entirely. A more substantial shear (e.g., from a flow chamber or in the ocean), may remove most remaining bacteria.

The prompt removal of most bacteria from both the PEG-modified surfaces exposed to shear indicates a lessened strength of adhesion when compared to both the acid washed glass and Intersleek® alternative surfaces. Also, both PEG-modified surfaces exhibited considerably less bacterial colonisation before shear was applied after 6 days compared to Intersleek®, and while bacterial colonisation was similar to that seen on acid washed glass, this surface failed to exhibit the anti-fouling properties that were displayed by the removal of bacteria from the PEG-surfaces demonstrated under shear. It is also interesting that compositional variations of the PSS-PEG surfaces result in different degrees of adhesion, or fouling. When a copolymer with a higher proportion of PEG methyl ether acrylate units was used (PSS-PEG-2, 50 PEG side chains for every 330 SS units), the resistance to the adhesion of marine organisms was considerably enhanced than when a copolymer with a lower proportion of PEG methyl ether acrylate units was used (PSS-PEG 2, 15 PEG side chains for every 1500 SS units). As such, it appears that increasing the proportion of PEG side chains provides an improvement in microbial resistance to adhesion.

Example 14 - Microscope observations on *Ulva* sp. (formerly *Enteromorpha*) settlement

High concentrations of zoospores inoculated above the surfaces discussed above (at the same zoospore concentration and from the same sample) show that zoospores can detect a favorable surface relatively quickly. With the gold and PSS-PEG 1 surfaces, few swimming zoospores are present in medium after 2 hours, with most settled. However, with the PSS-PEG 2 and PSS surfaces, large numbers of swimming zoospores are still unsettled

after 4 hours. Eventually, zoospores either settle or die. If another surface is available (i.e., glass or plastic petrie dish) zoospores appear to preferentially settle on it.

Example 15 - Flow chamber adhesion assays

Slides were placed in plastic quadriperm dishes and these were filled with sterile seawater. A 2mL aliquot of sterile seawater with cells was added to each quadriperm dish. The concentration of cell suspensions used in all experiments was consistent, as determined with a haemocytometer. Cells were allowed to settle for 6 h at 16°C under Sylvania 15W Cool White fluorescent lamps.

Slides were mounted into a fully turbulent flow chamber based on that previously described (Schultz et al 2000, A turbulent channel flow apparatus for the determination of the adhesion strength of microfouling organisms, Biofouling 15: 243). The flow was generated by a 0.72kW thermoplastic impeller (Davey PowerChief CR-150, operating on 240 V). The flow rate was varied via a two-way vacuum valve on the outlet side of the pump. Water is pumped from an opaque PVC tank filled to approximately 250 L with Instant Ocean® (Aquarium Systems, OH, USA) artificial sea water. An electronic digital flow meter (Great Plains Industries, Inc. Wichita, KS) situated downstream of the pump monitored flow rate through the system, with a measured accuracy of +/- 1.0 % of the flow rate displayed.

The slides were mounted onto the slide holder from quadriperm dishes filled with Instant Ocean®. The slide holder is resident in an open tank continuous to the flow channel, and is constantly in the aqueous environment. Slides were subjected to a flow rate of either 60 L min⁻¹ or 120 L min⁻¹ for 5 min. For each flow rate, three replicate assays were performed. As a control, fouling was analysed on three slides which had not been exposed to shear.

Following removal of slides from the flow chamber, autofluorescence images were obtained using a mercury bulb light source with an Olympus BH-2 epi-fluorescence microscope and a Leica DC 300F digital camera. For each slide, twenty images were obtained at random location under 40x magnification. Images were converted to an indexed colour mode using Adobe Photoshop 6.0.1 graphics editor software. In the case of diatoms, individual cells were then counted. In the case of Ulva, fluorescent pixels in each image, indicating zoospores, were counted and from this an estimation of percent area cover of fouling was determined (For detail of image analysis, see Molino et al. 2009, Development of the primary bacterial microfouling layer on antifouling and fouling release coatings in temperate and tropical environments in Eastern Australia, Biofouling, 1-14).

(a) Flow chamber adhesion assays of biofouling adhesion (*Haslea sp.*) to PVA/PEG_{EPOX} test surfaces: The mean number of *Haslea sp.* cells settled on all PVA/PEG_{EPOX} test surfaces was considerably lower than on the glass control (Fig. 24).

5 Following exposure to shear of 120 L min⁻¹, all settled cells had been removed from (PVA/PEG_{EPOX})₂ and (PVA/PEG_{EPOX})₃. This result is consistent with QCM data indicating negligible adhesion strength of *Haslea sp.*

(b) Flow chamber adhesion assays of biofouling adhesion (*Ulva sp. zoospores*) to PVA/PEG_{EPOX} test surfaces: While fouling on all (PVA/PEG_{EPOX}) surfaces was considerably lower than observed on glass control following exposure to shear, some residual fouling was observed (Fig. 25).

15 Finally, it will be appreciated that various modifications and variations of the methods and compositions of the invention described herein will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention that are apparent
20 to those skilled in the art are intended to be within the scope of the present invention.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of protecting a surface of an object from biological fouling, the method including:
 - 5 (a) providing a first functional group on the surface of the object;
 - (b) providing an anti-fouling composition containing a polymeric material, the polymeric material (i) containing side chains of polymeric moieties which inhibit biological fouling and (ii) containing a second functional group;
 - 10 (c) applying the anti-fouling composition to the surface of the object under conditions suitable for forming a bond between the first functional group and the second functional group.
- 15 2. A method according to claim 1 wherein the polymeric moieties which inhibit biological fouling include vinyl pyrrolidone units or ethylene oxide units.
3. A method according to claim 1 of claim 2 wherein providing a first functional group on the surface of the object includes activation of the surface.
- 20 4. A method according to claim 3 wherein activation of the surface includes oxidation of the surface.
5. A method according to claim 3 wherein activation of the surface includes acidification of the surface.
- 25 6. A method according to claim 3 wherein activation of the surface includes reaction of the surface with a chemical entity to provide a first functional group on the surface.
- 30 7. A method according to any one of claims 1 to 6 wherein providing a first functional group on the surface of the object includes applying to the surface a primer composition which includes a material containing the first functional group.
8. A method of protecting a surface of an object from biological fouling, the method including:
 - 35 (a) applying a primer composition including a material containing a first functional group to the surface of the object;

- (b) providing an anti-fouling composition containing a polymeric material, the polymeric material (i) having side chains containing one or more ethylene oxide units and (ii) containing a second functional group;
- (c) applying the anti-fouling composition to the surface of the object under conditions suitable for forming a bond between the first functional group and the second functional group.

9. A method according to claim 8 wherein the primer composition contains a polymeric material containing the first functional group.

10. A method according to any one of claims 1 to 9, wherein the first functional group is a hydroxy group.

11. A method according to claim 9 wherein the polymeric material containing the first functional group is poly(vinyl alcohol).

12. A method according to claim 8 or claim 9 wherein the primer composition contains a polymeric material containing a positively charged species selected from the group consisting of protonated primary amino, protonated secondary amino, protonated tertiary amino and quaternary ammonium groups, or a negatively charged species selected from the group consisting of carboxylate groups, sulfonate groups, sulfate groups and phosphonate groups.

13. A method according to claim 12 wherein the primer composition contains a polymeric material containing a positively charged species selected from the group consisting of protonated primary amino, protonated secondary amino, protonated tertiary amino and quaternary ammonium groups.

14. A method according to claim 12 wherein the primer composition contains a polymeric material containing a negatively charged species selected from the group consisting of carboxylate groups, sulfonate groups, sulfate groups and phosphonate groups.

15. A method according to claim 13 wherein the primer composition contains a polyamine.

16. A method according to claim 15 wherein the polyamine is selected from the group consisting of poly(ethyleneimine), poly(allylamine hydrochloride) and poly(vinylamine hydrochloride).

17. A method according to claim 9 wherein the primer composition contains a block, gradient, star or random copolymer of an acrylate with pendant azide groups and a polymerisable ester monomer of poly(ethylene glycol), or a block, gradient, star or random copolymer of an acrylate with pendant alkyne groups and a polymerisable ester monomer of poly(ethylene glycol).

18. A method according to claim 17 wherein copolymer is a random copolymer.

19. A method according to claim 17 wherein the primer composition contains a random copolymer of an acrylate with pendant azide groups and a polymerisable ester monomer of poly(ethylene glycol).

20. A method according to claim 17 wherein the primer composition contains a random copolymer of an acrylate with pendant alkyne groups and a polymerisable ester monomer of poly(ethylene glycol).

21. A method according to any one of claims 8 to 11, wherein the polymeric material in the anti-fouling composition contains epoxide groups.

22. A method according to claim 21, wherein the epoxide groups are located in side chains of the polymeric material in the anti-fouling composition.

23. A method according to any one of claims 8 to 11, 21 or 22, wherein the polymeric material in the anti-fouling composition is a copolymer of glycidyl acrylate and/or glycidyl methacrylate and a polymerisable ester monomer of poly(ethylene glycol).

24. A method according to any one of claims 1 to 9 wherein the anti-fouling composition contains a polymeric material containing a positively charged species selected from the group consisting of protonated primary amino, protonated secondary amino, protonated tertiary amino and quaternary ammonium groups, or a negatively charged species selected from the group consisting of carboxylate groups, sulfonate groups, sulfate groups and phosphonate groups.

25. A method according to claim 24 wherein the anti-fouling composition contains a polymeric material containing a positively charged species selected from the group consisting of protonated primary amino, protonated secondary amino, protonated tertiary amino and quaternary ammonium groups.

26. A method according to claim 24 wherein the anti-fouling composition contains a polymeric material containing a negatively charged species selected from the group consisting of carboxylate groups, sulfonate groups, sulfate groups and phosphonate groups.

5

27. A method according to any one of claims 1 to 9 and 13, 15 or 16 wherein the anti-fouling composition contains a copolymer prepared by polymerization of a polymerisable ester monomer of poly(ethylene glycol) and a sulfonated ethylenically unsaturated monomer.

10 28. A method according to claim 27 wherein the sulfonated ethylenically unsaturated monomer is 4-styrene sulfonate.

29. A method according to claim 28 wherein the anti-fouling composition contains a block copolymer of sodium 4-styrenesulfonate and poly(ethylene glycol) methyl ether acrylate.

15

30. A method according to any one of claims 1 to 9 and 19 wherein the anti-fouling composition contains a random copolymer of an acrylate with pendant alkyne groups and a polymerisable ester monomer of poly(ethylene glycol).

20 31. A method according to any one of claims 1 to 9 and 20 wherein the anti-fouling composition contains a random copolymer of an acrylate with pendant azide groups and a polymerisable ester monomer of poly(ethylene glycol).

25 32. A method according to claim any one of claims 17, 19, 20, 23, 27, 30 or 31 wherein the polymerisable ester monomer is selected from the group consisting of poly(ethylene glycol) acrylates, poly(ethylene glycol) methacrylates, poly(ethylene glycol) methyl ether acrylates and poly(ethylene glycol) methyl ether methacrylates.

30 33. A method according to any one of claims 1 to 32, wherein the each side chain contains between 1 and 1000 ethylene oxide units.

34. A method according to any one of claims 1 to 32, wherein each side chain contains between 1 and 100 ethylene oxide units.

35 35. A method according to any one of claims 1 to 32, wherein each side chain contains between 1 and 20 ethylene oxide units.

36. A method according to any one of claims 1 to 32, wherein each side chain contains between 3 and 12 ethylene oxide units.

37. A method according to any one of claims 1 to 36, wherein the object is a ship, boat, yacht, dinghy, submarine, ferry, speedboat, frigate, aircraft carrier, minesweeper, ice breaker, rowboat, skiff, jet ski, ketch, yawl, sloop, schooner, tanker, barge, or tugboat.

38. A method according to any one of claims 1 to 36, wherein the object is a surfboard, boogie board, windsurfer or waterski.

39. A method according to any one of claims 1 to 36, wherein the object is a buoy, pier, wharf, jetty, beacon, breakwater, oil rig, fish farm fence, shark net, fishing net, pontoon, crab pot, lobster pot, shark cage or dock.

40. A method according to any one of claims 1 to 36, wherein the object is a keel, fin, mast, sail, rudder, propeller, periscope or cabin.

41. A method according to any one of claims 1 to 36, wherein the object is the glass surface of a domestic or commercial aquaria, an ornamental rock or statue, an underwater viewing vessel, a glass bottomed boat, or a semi-submersible.

42. A method according to any one of claims 1 to 36, wherein the object is a material or piece of equipment used in industrial processing.

43. A method according to any one of claims 1 to 36, wherein the object is a material or piece of equipment used in small scale processing.

44. A method according to claim 42 or claim 43, wherein the material or piece of equipment is in constant, frequent or intermittent contact with aqueous solutions.

45. A method according to any one of claims 42 to 44, wherein the material or piece of equipment is used in systems for power generation, water treatment, desalinisation or food processing.

46. A method according to any one of claims 42 to 45, wherein the material is selected from the group consisting of reverse osmosis membranes and particulate filters.

47. A method according to any one of claims 42 to 45, wherein the piece of equipment is selected from the group consisting of water intakes, piping systems, condensers, evaporators, valves, inlets, outlets and heat exchangers.

5 48. A method according to any one of claims 1 to 47, wherein the steps (a) to (c) are repeated n times, wherein n is between 1 and 100.

49. An object containing a surface protected by a method according to any one of claims 1 to 48.

10

50. A kit for use in protecting a surface from biological fouling the kit including:

- (a) a primer composition including a material containing a first functional group;
- (b) an anti-fouling composition containing a polymeric material, the polymeric material (i) containing side chains of polymeric moieties which inhibit biological fouling and (ii) containing a second functional group;

15

wherein the first functional group and the second functional group form a bond once the two compositions have been applied to the surface.

51. A kit according to claim 50, wherein the polymeric moieties which inhibit biological fouling are chosen from vinyl pyrrolidone units and ethylene oxide units.

20

52. A kit according to claim 50 or claim 51 wherein the primer composition contains a polymeric material containing the first functional group.

25 53. A kit according to any one of claims 50 to 52, wherein the first functional groups are hydroxy groups.

54. A kit according to claim 52 wherein the polymeric material containing the first functional group is poly(vinyl alcohol).

30

55. A kit according to claim 50 or claim 51 wherein the primer composition contains a polymeric material containing a positively charged species selected from the group consisting of protonated primary amino, protonated secondary amino, protonated tertiary amino and quaternary ammonium groups, or a negatively charged species selected from the group consisting of carboxylate groups, sulfonate groups, sulfate groups and phosphonate groups.

35

56. A kit according to claim 55 wherein the primer composition contains a polymeric material containing a positively charged species selected from the group consisting of protonated primary amino, protonated secondary amino, protonated tertiary amino and quaternary ammonium groups.

5

57. A kit according to claim 55 wherein the primer composition contains a polymeric material containing a negatively charged species selected from the group consisting of carboxylate groups, sulfonate groups, sulfate groups and phosphonate groups.

10 58. A kit according to claim 56 wherein the primer composition contains a polyamine.

59. A kit according to claim 58 wherein the polyamine is selected from the group consisting of poly(ethyleneimine), poly(allylamine hydrochloride) and poly(vinylamine hydrochloride).

15

60. A kit according to claim any one of claims 50 to 52 wherein the primer composition contains a block, gradient, star or random copolymer of an acrylate with pendant azide groups and a polymerisable ester monomer of poly(ethylene glycol), or a block, gradient, star or random copolymer of an acrylate with pendant alkyne groups and a polymerisable ester monomer of poly(ethylene glycol).

20

61. A kit according to claim 60 wherein the copolymer is a random copolymer.

62. A kit according to claim 61 wherein the primer composition contains a random copolymer of an acrylate with pendant azide groups and a polymerisable ester monomer of poly(ethylene glycol).

25

63. A kit according to claim 61 wherein the primer composition contains a random copolymer of an acrylate with pendant alkyne groups and a polymerisable ester monomer of poly(ethylene glycol).

30

64. A kit according to any one of claims 50 to 54, wherein the wherein the polymeric material in the anti-fouling composition contains epoxide groups.

35 65. A kit according to claim 64, wherein the epoxide groups are located in side chains of the polymeric material in the anti-fouling composition.

66. A kit according to any one of claims 50 to 54, 64 or 65, wherein the polymeric material in the anti-fouling composition is a copolymer of glycidyl acrylate and/or glycidyl methacrylate and a polymerisable ester monomer of poly(ethylene glycol).

5 67. A kit according to claim 50 or 51 wherein the anti-fouling composition contains a polymeric material containing a positively charged species selected from the group consisting of protonated primary amino, protonated secondary amino, protonated tertiary amino and quaternary ammonium groups, or a negatively charged species selected from the group consisting of carboxylate groups, sulfonate groups, sulfate groups and phosphonate groups.

10 68. A kit according to claim 67 wherein the anti-fouling composition contains a polymeric material containing a positively charged species selected from the group consisting of protonated primary amino, protonated secondary amino, protonated tertiary amino and quaternary ammonium groups.

15 69. A kit according to claim 67 wherein the anti-fouling composition contains a polymeric material containing a negatively charged species selected from the group consisting of carboxylate groups, sulfonate groups, sulfate groups and phosphonate groups.

20 70. A kit according to any one of claims 50 to 52, 56 and 58 to 59 wherein the anti-fouling composition contains a copolymer prepared by polymerization of a polymerisable ester monomer of poly(ethylene glycol) and a sulfonated ethylenically unsaturated monomer.

25 71. A kit according to claim 70 wherein the sulfonated ethylenically unsaturated monomer is 4-styrene sulfonate.

72. A kit according to claim 71 wherein the anti-fouling composition contains a block copolymer of sodium 4-styrenesulfonate and poly(ethylene glycol) methyl ether acrylate.

30 73. A kit according to any one of claims 50 to 52 or 62 wherein the anti-fouling composition contains a random copolymer of an acrylate with pendant alkyne groups and a polymerisable ester monomer of poly(ethylene glycol).

35 74. A kit according to any one of claims 50 to 52 or 63 wherein the anti-fouling composition contains a random copolymer of an acrylate with pendant azide groups and a polymerisable ester monomer of poly(ethylene glycol).

75. A kit according to claim any one of claims 60, 62, 63, 66, 70, 73 or 74 wherein the polymerisable ester monomer is selected from the group consisting of poly(ethylene glycol) acrylates, poly(ethylene glycol) methacrylates, poly(ethylene glycol) methyl ether acrylates and poly(ethylene glycol) methyl ether methacrylates.

5

76. A method according to claim 1 substantially as hereinbefore described with reference to any one of the examples.

Figure 1

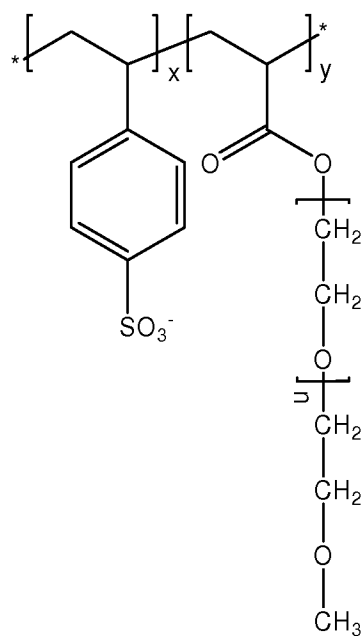
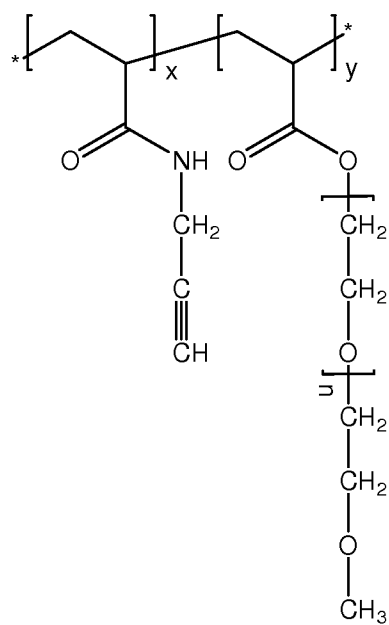
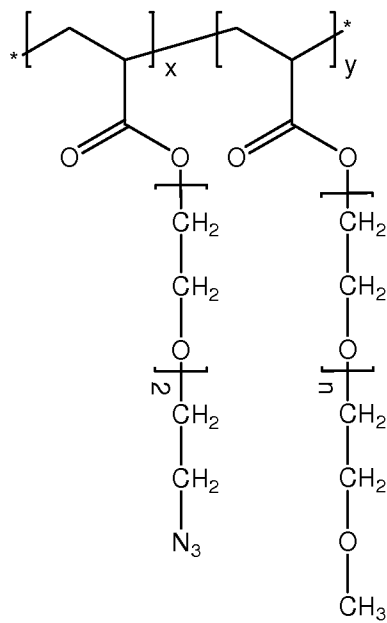


Figure 2



(1) Click-PEG-Alk



(2) Click-PEG-Az

Figure 3

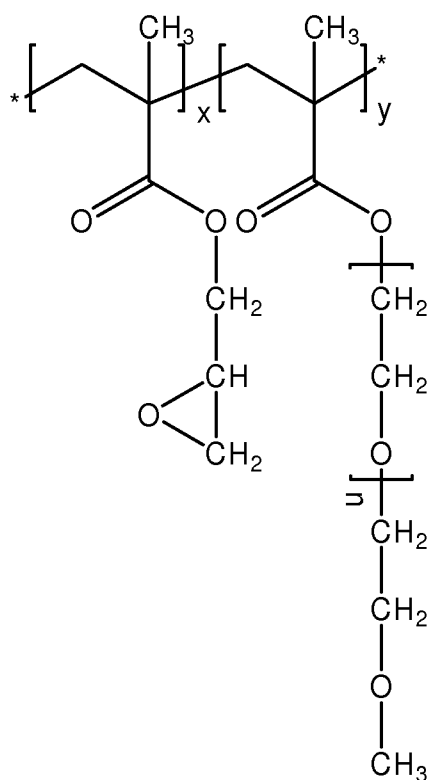


Figure 4

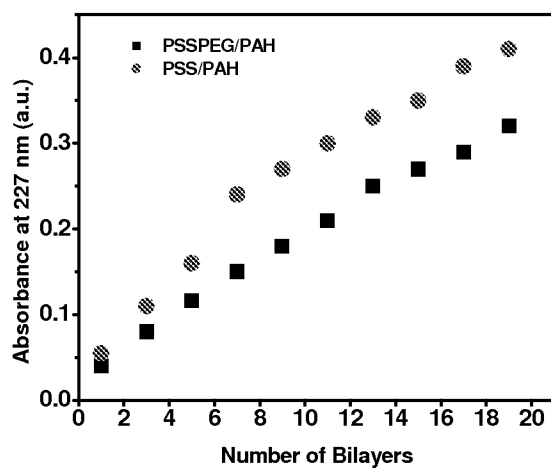


Figure 5

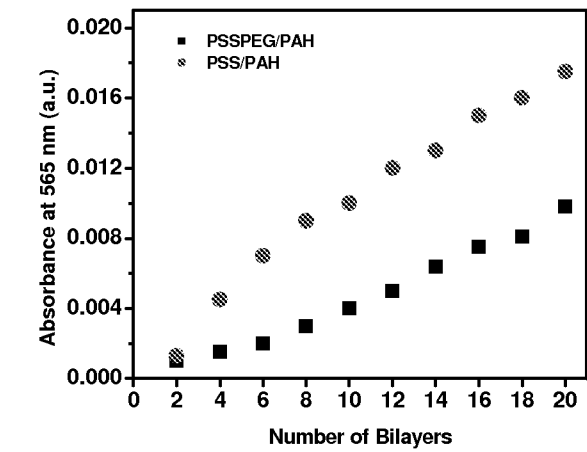


Figure 6

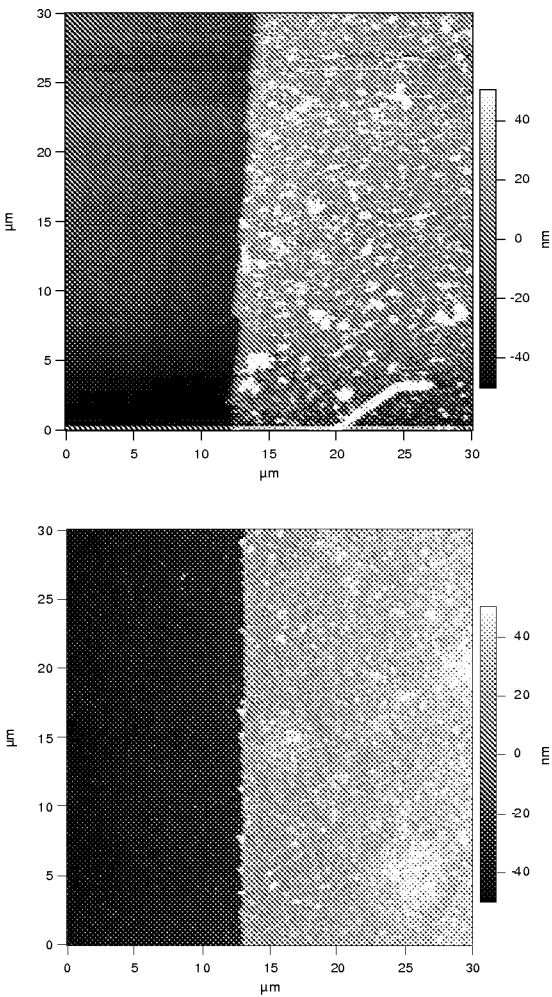


Figure 7

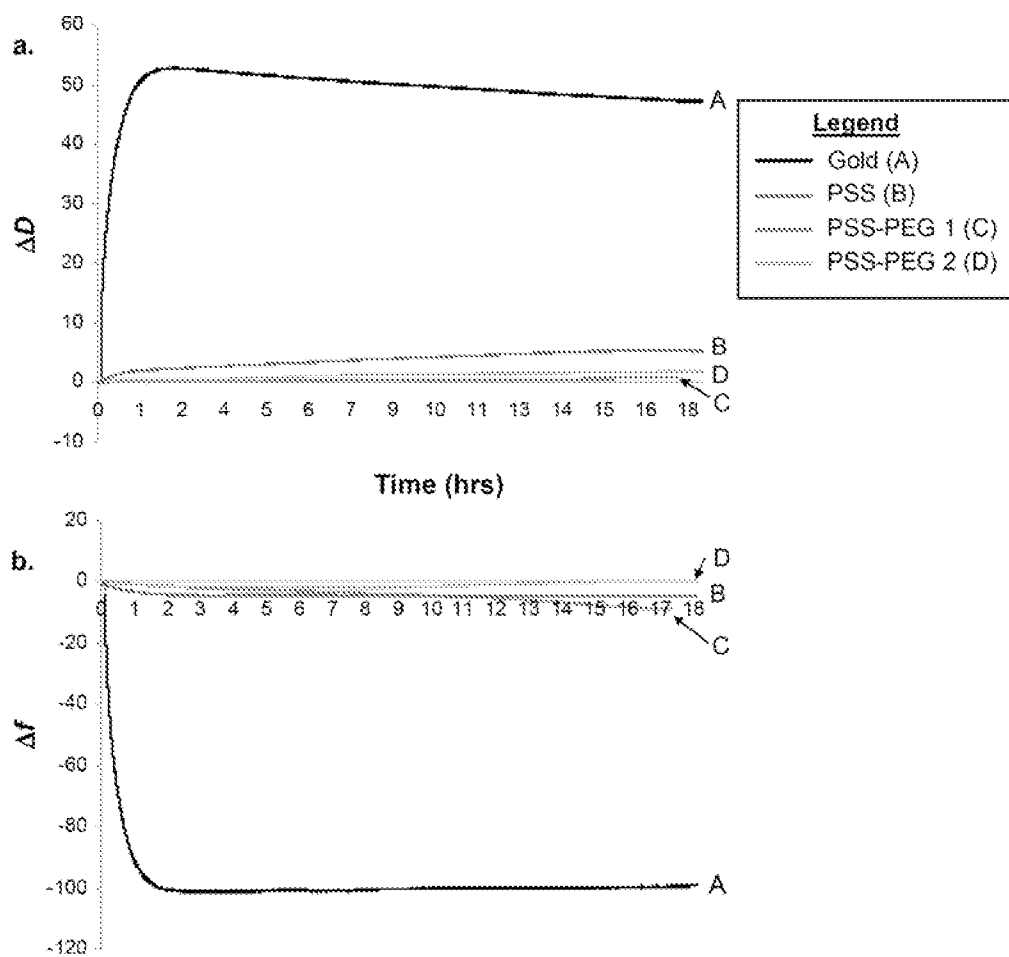


Figure 8

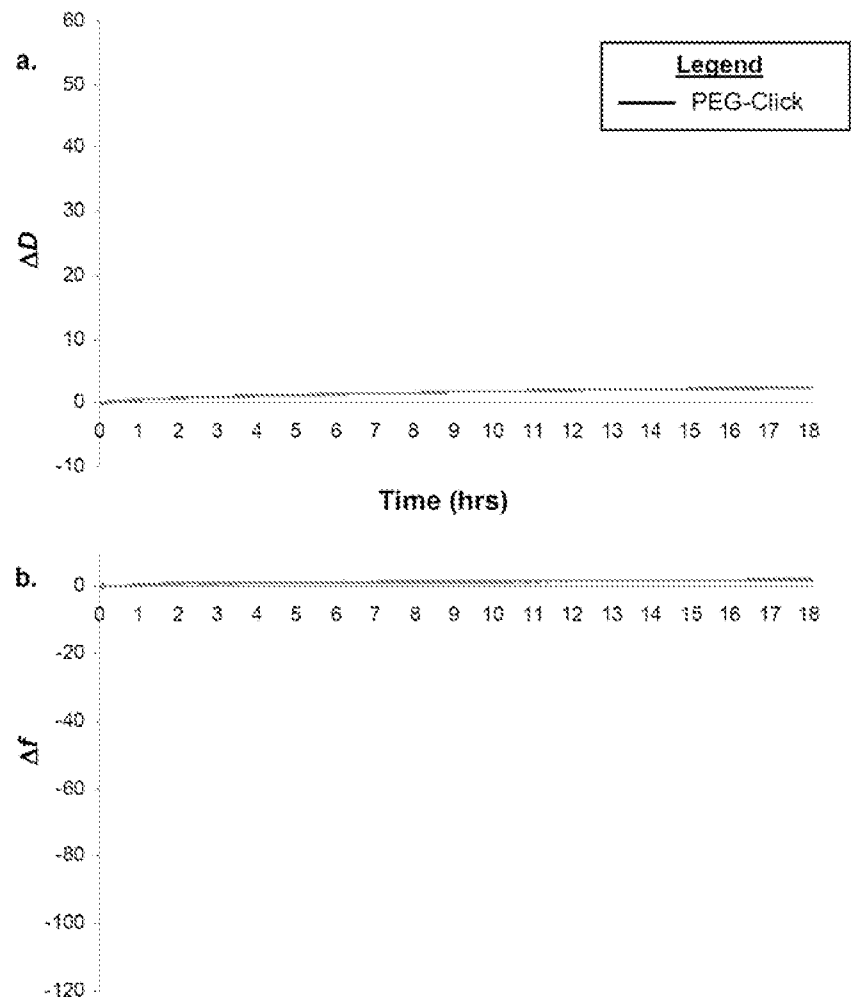


Figure 9

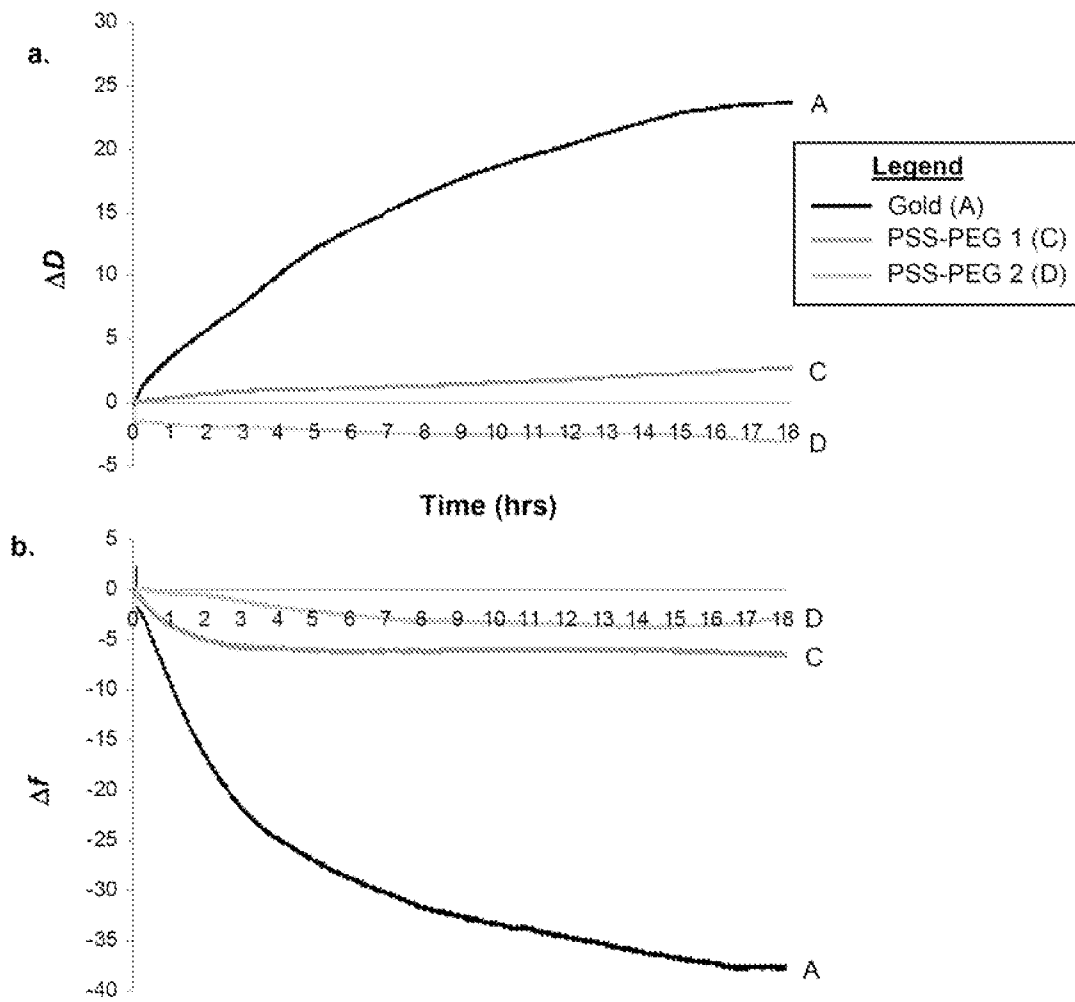


Figure 10

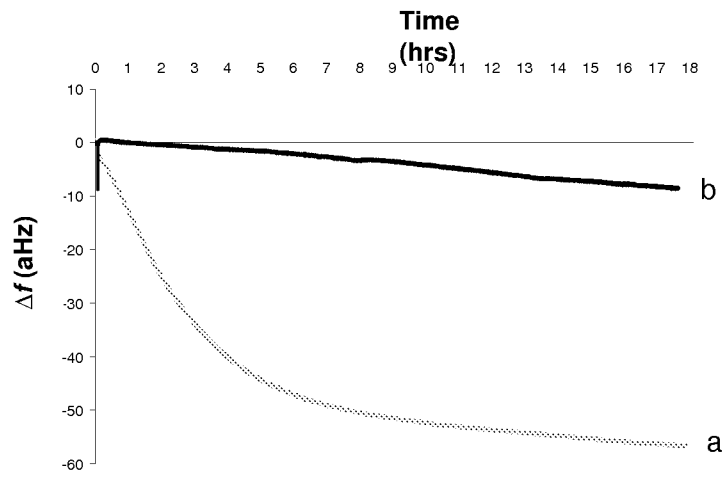


Figure 11

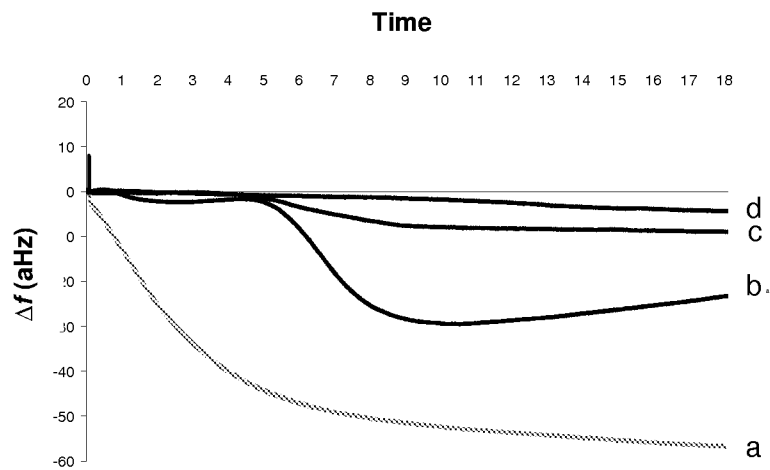


Figure 12

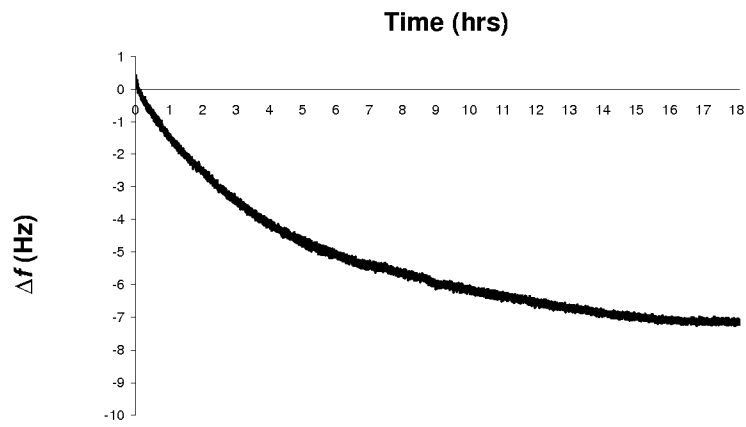


Figure 13

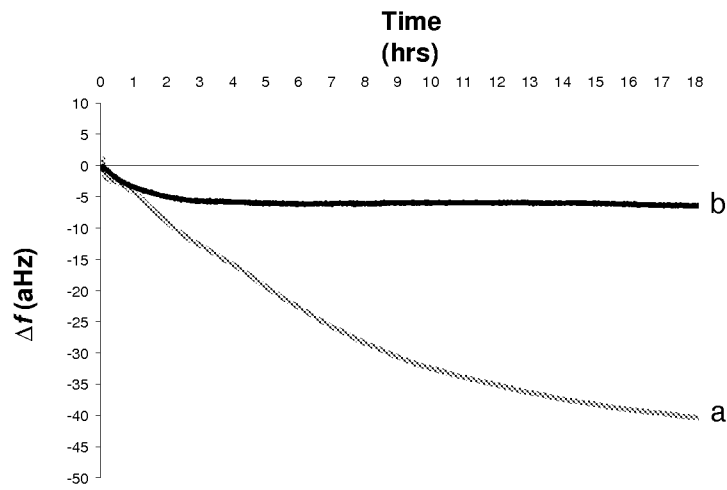


Figure 14

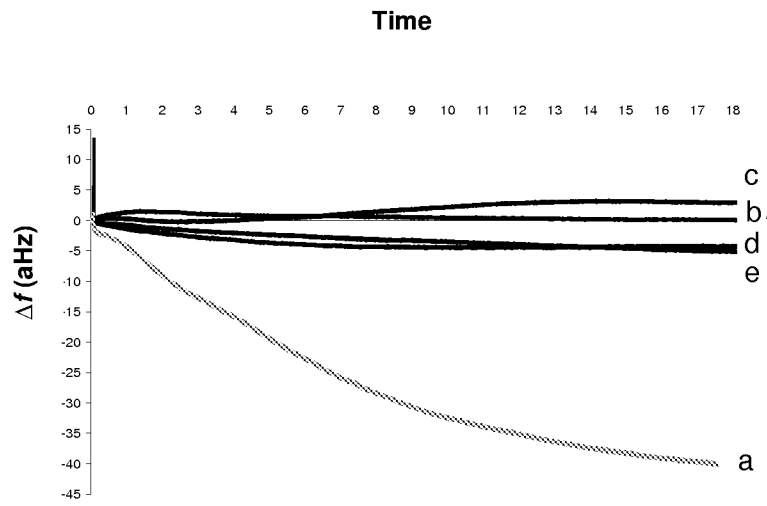


Figure 15

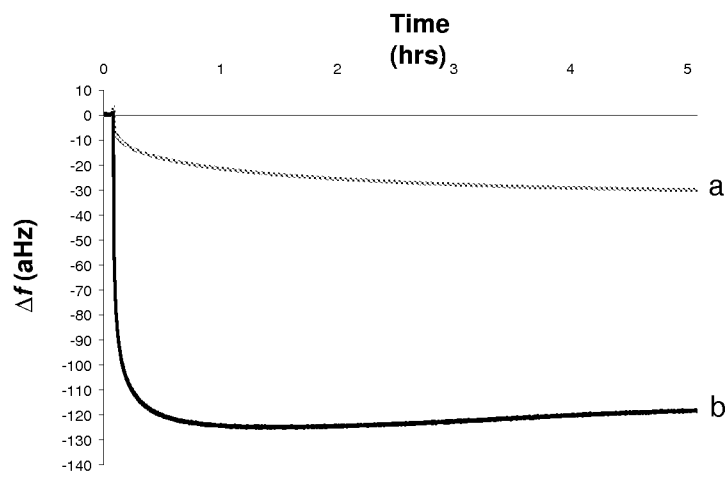


Figure 16

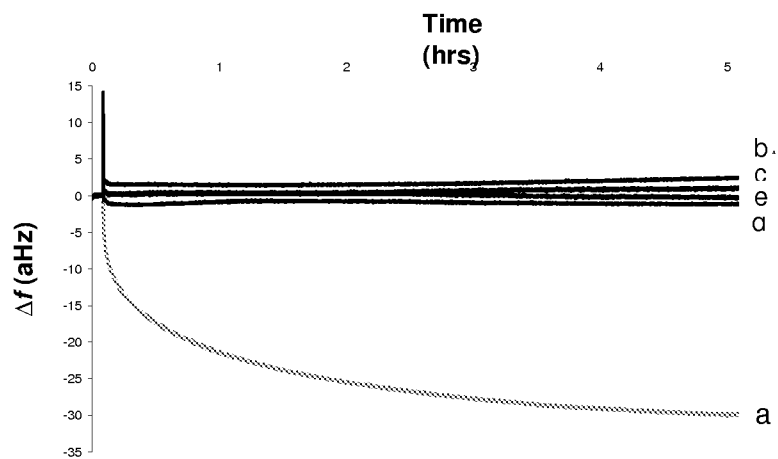


Figure 17

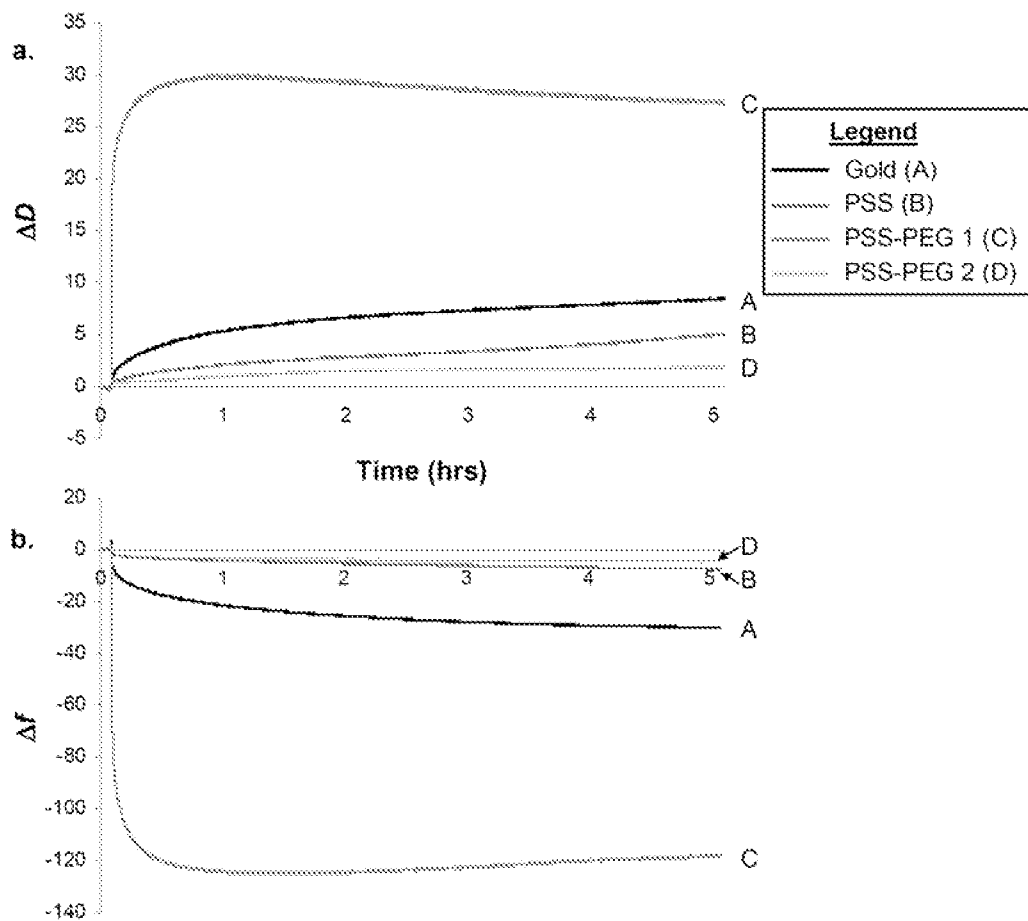


Figure 18

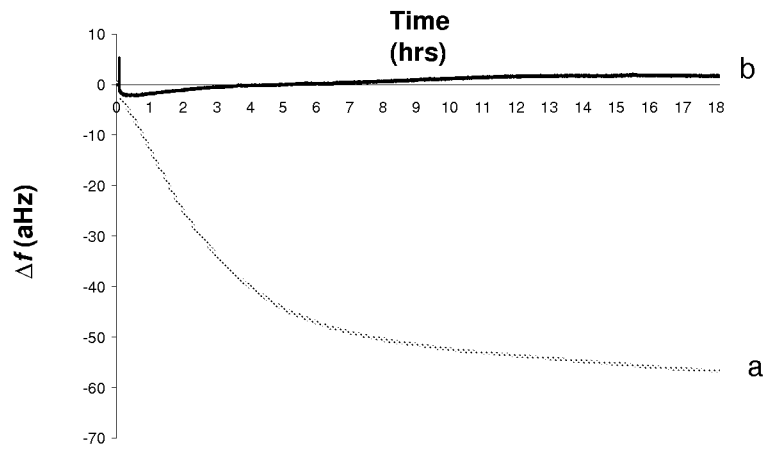


Figure 19

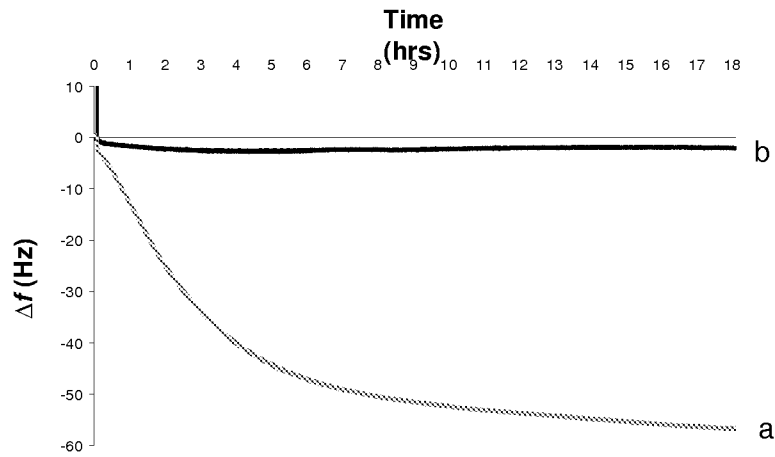


Figure 20

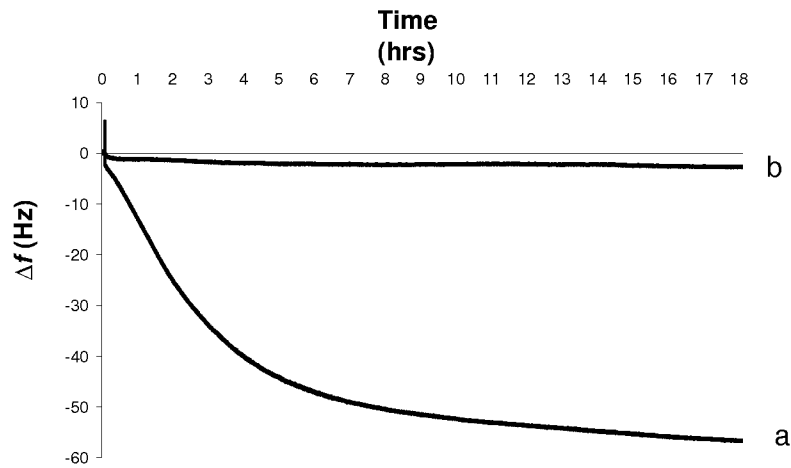


Figure 21

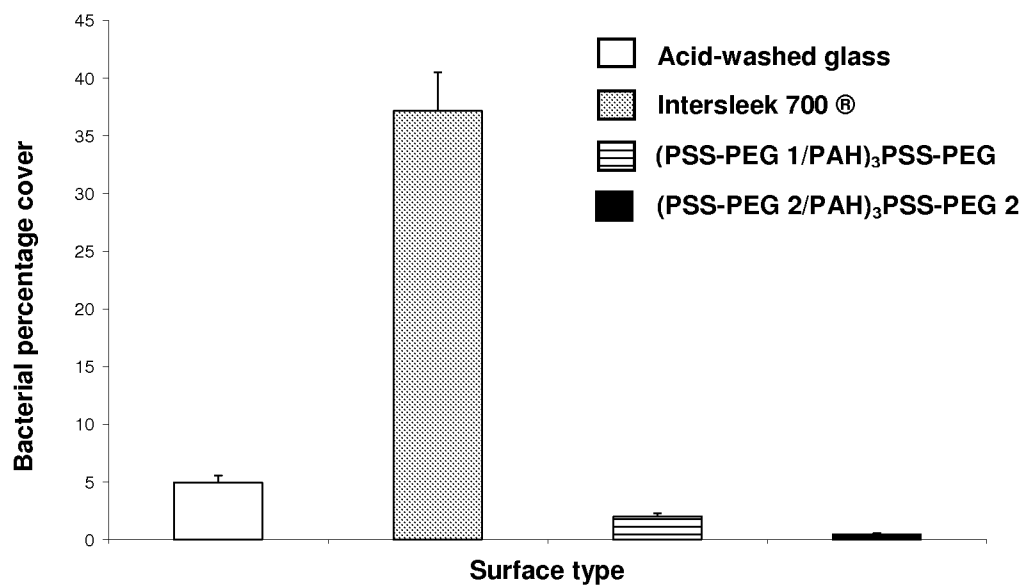


Figure 22

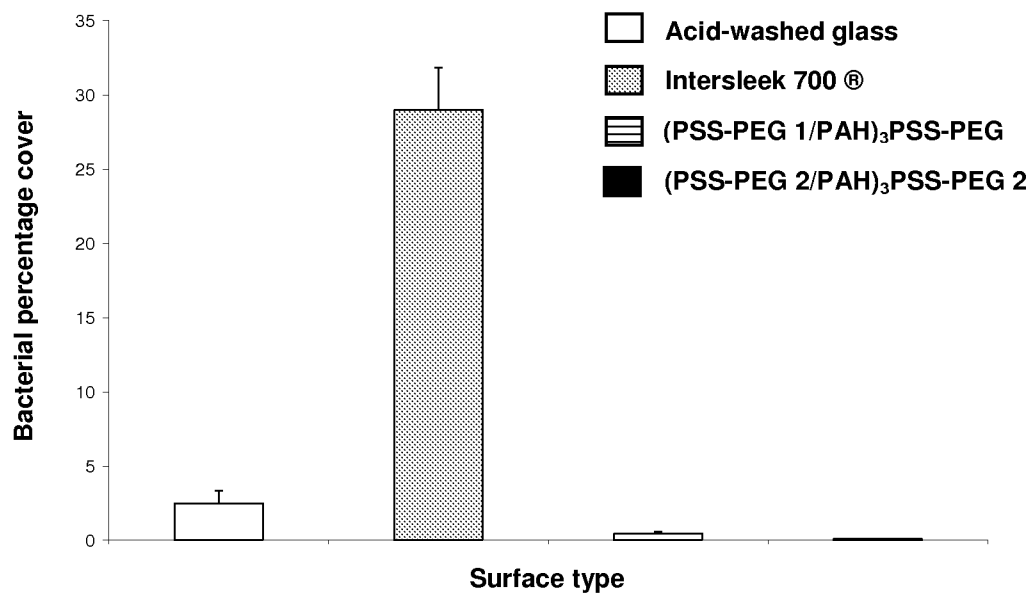


Figure 23

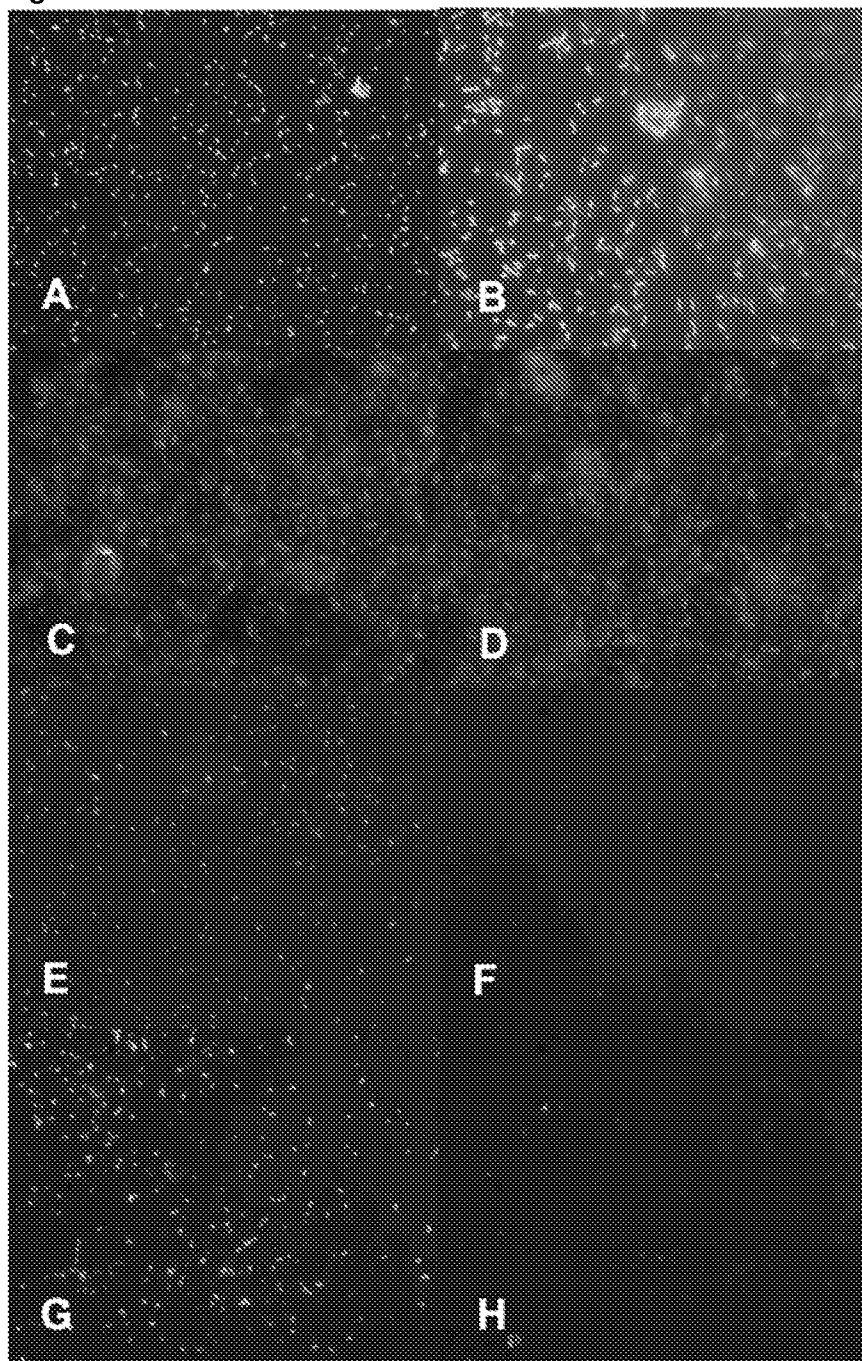


Figure 24

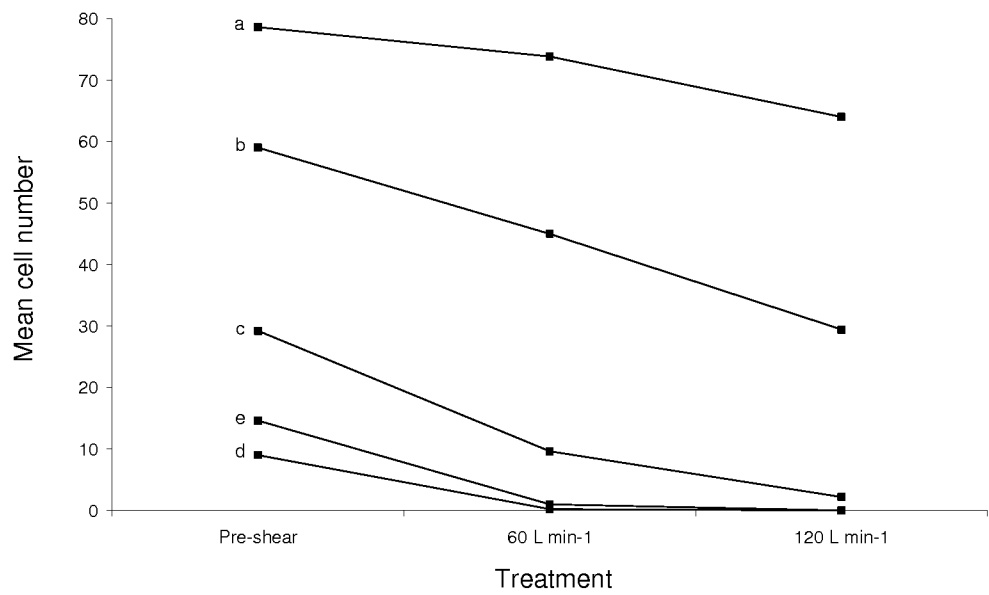
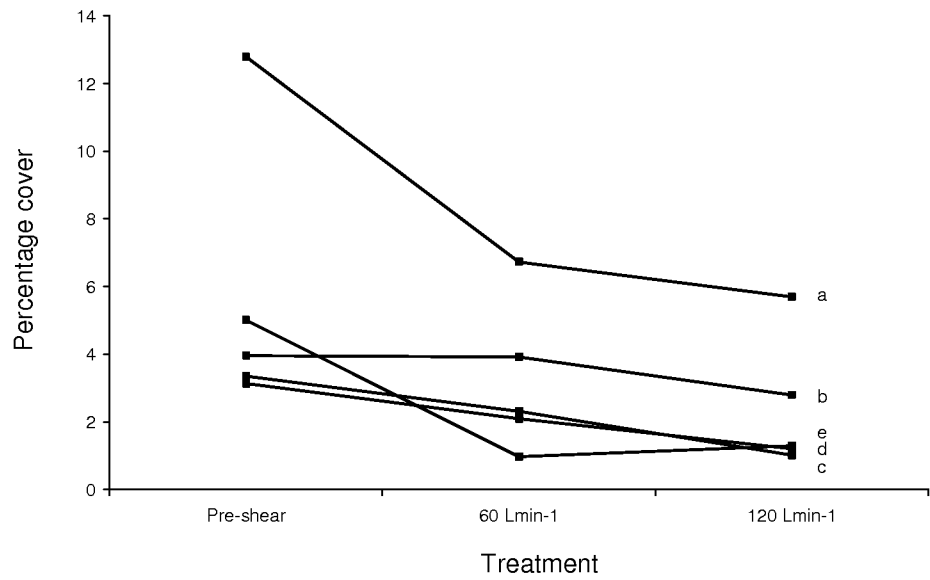


Figure 25



INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2008/001869

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

C09D 5/16 (2006.01)

B08B 17/00 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, EPODOC, CAPLUS: Keywords (ANTIFOULING, FOULING PROTECTION, PRIMER, UNDERCOAT, PRECOAT) and similar terms.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP 2004-202385 A (CHUGOKU MARINE PAINTS LTD & CHUBU ELECTRIC POWER CO INC) 22 July 2004 [Patent Abstracts of Japan - Machine translation] [0012-0019, 0077, 0147-0160]	1-2, 6-10, 12-14, 21-22, 24-26, 33-75
X,Y		3-5



Further documents are listed in the continuation of Box C



See patent family annex

* "A"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier application or patent but published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
23 January 2009Date of mailing of the international search report
13 FEB 2009Name and mailing address of the ISA/AU
AUSTRALIAN PATENT OFFICE
PO BOX 200, WODEN ACT 2606, AUSTRALIA
E-mail address: pct@ipaustalia.gov.au
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(ISO 9001 Quality Certified Service)
Telephone No : +61 2 6283 2744

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2008/001869

C (Continuation).

DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,Y	WO 2003/080747 A1 (JOTUN AS) 2 October 2003 page 3 lines 3-27, page 23 lines 23-32, page 27 line 26 to page 28 line 27, and Table 3	1-7, 10, 37-46, 49-53
X,Y	WO 2002/000785 A1 (DUPONT TEIJIN FILMS US LIMITED PARTNERSHIP) 3 January 2002 page 3 lines 1-6, page 18 lines 1-26, page 21 lines 11-16	1-7, 10, 24-26 37-47, 49-53, 67-69
X,Y	US 2007/0129461 A1 (FYLES) 7 June 2007 Abstract, 0054-0059, 0063, 0084	1, 3-7, 10, 24-26, 37-47, 49-50, 52-53, 67-69
X,Y	EP 040498 A1 (M & T CHEMICALS B.V.) 25 November 1981 Abstract, page 3 line 15 to page 4 line 13, and page 6 to page 7	1, 3-7, 10, 24-26, 37-47, 49-50, 52-53, 67-69
X,Y	US 2007/0021529 A1 (BOUDJOUK ET AL) 25 January 2007 Abstract, 0094, Figures 1-3	1, 3-7, 24-25, 37-47, 49-50, 52, 67-68

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2008/001869

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 76
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claim 76 does not comply with Rule 6.2(a) because it relies on references to the description.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

[See Supplemental Box I]

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2008/001869

Supplemental Box I

(To be used when the space in any of Boxes I to IV is not sufficient)

Continuation of Box No: III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept. This International Searching Authority has found that there are different inventions as follows:

- Claims 1 to 49 are directed to a method of protecting a surface from biological fouling which is considered to comprise a first distinguishing feature.
- Claims 50-75 are directed to components (a) and (b) which are merely suitable for use in protecting a surface from biological fouling which is considered that to comprise a second distinguishing feature.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

Given that the term “kit for use in protecting a surface from biological fouling” (emphasis added) is considered to encompass the use of components (a) and (b) for applications other than “protecting a surface from biological fouling”. The only feature common to all of the claims is components (a) and (b) which are merely suitable for use in protecting a surface from biological fouling. However this concept is not novel in the light of:

D1. JP 2004-202385

This means that the common feature can not constitute a special technical feature within the meaning of PCT Rule 13.2, second sentence, since it makes no contribution over the prior art. Because the common feature does not satisfy the requirement for being a special technical feature it follows that it cannot provide the necessary technical relationship between the identified inventions. Therefore the claims do not satisfy the requirement of unity of invention *a posteriori*.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/AU2008/001869

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member			
JP	2004202385	JP	4060701		
WO	03080747	AU	2003225433	CN	1643085
		NO	20021491	AT	404642
		JP	2005520912	EP	1487928
				DE	60322853
WO	0200785	CN	1441828	EP	1297068
		AT	335045	CN	1216937
		GB	0015622	JP	2004502011
		US	2004009361	US	6743520
				DE	60121955
				TW	570949
US	2007129461	NONE			
EP	0040498	AR	228600	AU	70277/81
		ES	8206571	GR	75258
		IL	62825	KR	840001607
		NL	8002689	NO	811565
		PT	72998	ZA	8103083
				BR	8102882
				JP	57004905
				ES	502025
				NZ	197037
US	2007021529	EP	1663531	WO	2005030405
				JP	2007521381
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.					
END OF ANNEX					