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Supporting Information

Antibody-Functionalized Polydiacetylene Coatings on Nanoporous Membranes for Microorganism Detection

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Materials. 10,12-pentacosadiynoic acid (PCDA) and 10,12-tricosadiynoic acid (TRCDA) were obtained from GFS Chemicals (Powell, OH). Bovine polyclonal antibodies to *Cryptosporidium parvum* were obtained from the NIH AIDS Reagents Program, originally donated by Dr. Joseph Crabb. Goat anti-*E. coli* were obtained from Virostat, Inc. (Portland, Maine). N-(6-Maleimidohexanoyl)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine, sodium salt (MX-DSPE) was obtained from Northern Lipids, Inc. (Vancouver, British Colombia). CDP star chemiluminescent alkaline phosphatase (AP) substrate was purchased from EMD Biosciences. 1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine, 4-chlorobenzenesulfonate salt ($DiIC_{18}(5)$) was obtained from Molecular Probes (Eugene, OR). All other reagents and solvents were obtained from commercial suppliers and were used without further purification. *Escherichia coli* were purchased from the American Type Culture Collection (ATCC #23716) and were propagated in Lennox LB broth at 37 °C with 220 RPM shaking. 0.2 μm cellulose nitrate (CN) and 0.2 μm Nylon membranes are Whatman brand. 0.22 μm polycarbonate (PC) membranes are Osmonics brand. 0.05 μm and 0.45 μm mixed cellulose esters (MCE) and 0.1 μm polyvinylidene difluoride (PVDF) membranes are Millipore brand. All membranes and filter plates were purchased from Fisher Scientific.

Antibody-tail Conjugates. Antibodies were prepared for modification with an amphiphilic tail by reduction of hinge disulfides or generation of surface thiols. Hinge reduction was accomplished by combining 1.7 mL antibody (1.8 mg/mL) with 65 μL N_2-sparged 58 mM mercaptoethanolamine (MEA) / 66 mM EDTA (200 equivalents) and rocking at 37 °C for 1.5 h. The solution was dialyzed (10K MWCO membrane) against Ar-sparged 5 mM NaOAc / 50 mM NaCl / 10 mM EDTA pH 4.7 (3 changes of 1 L) for 25 h. Surface thiols were generated by combining 1.7 mL antibody (1.8 mg/mL) with 10 μL 9.4 mM (5 equivalents) 2-iminothiolane in 10 mM $NaPO_4$ / 138 mM NaCl / 2.7 mM KCl pH 7.4 (PBS) and 75 μL 1 M $NaHCO_3$. The solution was shaken at room temperature for 1.5 h. It was then dialyzed (10K MWCO) against Ar-sparged PBS with 10 mM EDTA (3 changes of 1 L each) for 25 h.

The free thiols on the antibody were then reacted with the maleimide-containing tail MX-DSPE to generate the antibody-tail conjugate. 150 μL 5 mM MX-DSPE in ethanol (20 equivalents) was added to each of the thiol-containing antibodies and they were shaken at 37 °C for 16 h. Both antibody-tail conjugates were dialyzed (10K MWCO) against PBS (3 changes of 1.2 L) for 7.5 h. They were stored at 4 °C until use.

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Coating Preparation. Organic solutions of the desired coating components were combined and dried down with a stream of N_2 followed by rotary evaporation. Water or buffer was added to the solid and the mixture sonicated with a probe sonicator to obtain a transparent or translucent suspension. The colloidal suspensions were chilled overnight at 10 °C. The colloids were combined with a 20:1 weight ratio of antibody-tail conjugate and 0.6% deoxycholate. The detergent was removed by dialysis with a 10K MWCO membrane against a 1 mM $NaPO_4$ / 1.5 mM KPO_4 / 10 mM KCl pH 7.1 buffer.

Free membranes or filter plates were used untreated or were treated with poly-D-lysine (1 mg/mL) for 3 h and allowed to dry. The colloids were deposited on the membranes by filtration and chilled overnight. Coatings are deposited onto free-standing membranes via positive pressure using a syringe and filter holder. Deposition on membranes in 96-well filter plates is accomplished by negative pressure using a vacuum manifold. Coatings were then polymerized with 254 nm UV light until they were blue.

Immuno-TEM. Samples to be observed were fixed in 0.1 M phosphate buffer with 4% formalin at pH 7.4 and shipped to Paragon Bioservices. There they were treated with biotinylated anti-bovine antibody followed by incubation with 6 nm gold particles conjugated to streptavidin. The samples were then immersed in 0.2 M sucrose and post-fixed with 1% osmium tetroxide. Following fixation, the samples were dehydrated through graded alcohols and propylene oxide, and incubated in Epon and propylene oxide. They were then embedded in beam capsules and the resulting blocks were trimmed and sections cut. The sections were mounted and stained with uranyl and lead citrate. The stained grids were observed with a Zeiss EM10A/EM10B at 80kV and pictures taken.

Enzyme Immunoassays (EIA). Samples for EIA were initially blocked with 1% BSA in 50 mM Tris / 138 mM NaCl / 2.7 mM KCl pH 7.4 (TBS) with 0.2% Tween-20 pH 7.4 (TBS-tween) for 1 hour. The samples were washed with TBS-tween followed by treatment for 1 hour with an appropriate secondary antibody AP conjugate at 3,000 fold dilution of the stock in TBS. The samples were washed with TBS, and the chemiluminescent AP substrate CDPstar added. The samples were incubated for 15 minutes and their luminescence read in a Wallac Victor IIV. A calibration curve for the secondary antibody dilution and the substrate was generated for each experiment using a serial dilution series of the secondary antibody. The secondary antibody dilutions were combined with luminescent substrate and incubated for 15 minutes and their luminescence read as above. The calibration curve was used to calculate a relative amount of secondary antibody bound to the coatings for use in comparison of data between experiments. The kinetics of the solution and interfacial reactions are almost certainly different, however, so while the amounts of secondary antibody used in the calibration curve are known, only relative amounts of secondary antibody bound for the coatings can be reported.

Fluorescence Stability. Circles of 0.05 μm MCE, 0.2 μm CN, 0.2 μm Nylon, and 0.22 μm PC membranes were punched out and tacked with rubber cement to the well bottoms of a 96-well filter plate from which the original membranes had been removed.

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The modified plate was allowed to cure for 16 h at room temperature. TRCDA liposomes were deposited in the wells of the modified plate by vacuum filtration. The fluorescence emission at 642 nm was read in a Wallac Victor IIV multilabel plate reader, with excitation at 470 nm. The emission from the coatings was monitored periodically over the course of the following year.

***E. coli* Assay.** Samples for fluorescence were prepared in a 96-well filter plate with 0.45 μm MCE membranes. Fluorescence emission of the coatings was read in a Wallac Victor IIV multilabel plate reader, with excitation at 470 nm and measurement of the emission at 675 nm. Dilutions of *E. coli* were prepared in water. Solutions of *E. coli* (250 μL/well) were added to two wells and blank solutions to the other two wells. The solutions were filtered through the coatings and the emission read again. This process was repeated iteratively with increasing concentrations of *E. coli* in the first two wells and blank solutions in the other two wells. Four iterations were done with 125 organisms, four iterations with 1250 organisms, and four iterations with 12,500 organisms per well. As the PDA coating converted to the fluorescent form, the emission at 675 nm increases due to resonance energy transfer from the polymer to the fluorophore.