Antibody Catalyzed Water Oxidation Pathway Biosensor

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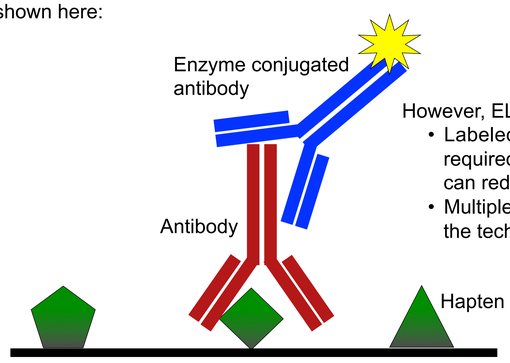
Introduction: Infectious Disease Detection

Infectious diseases, such as influenza, present a significant global challenge through the constant threat of pandemics. Early discovery of infection is often most easily accomplished by the detection of antibodies. The current standard method for antibody detection is based on the enzyme-linked immunosorbent assay (ELISA) as shown here:

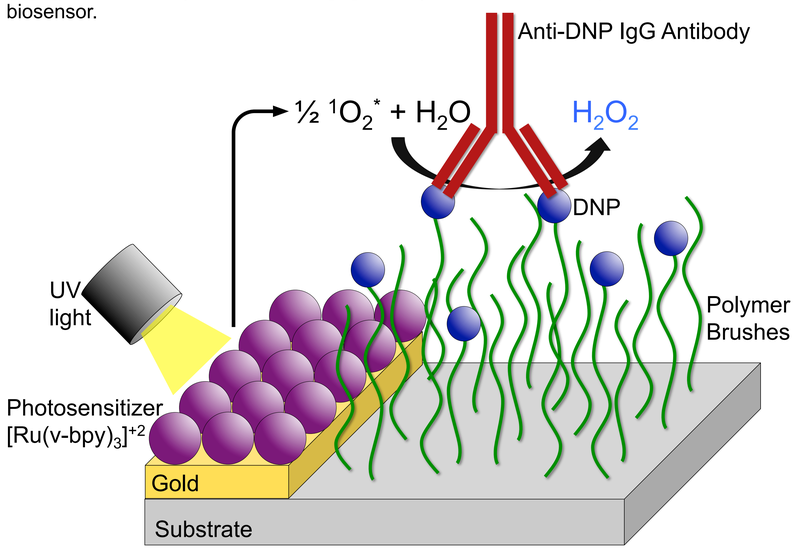


However, ELISA has several limitations:

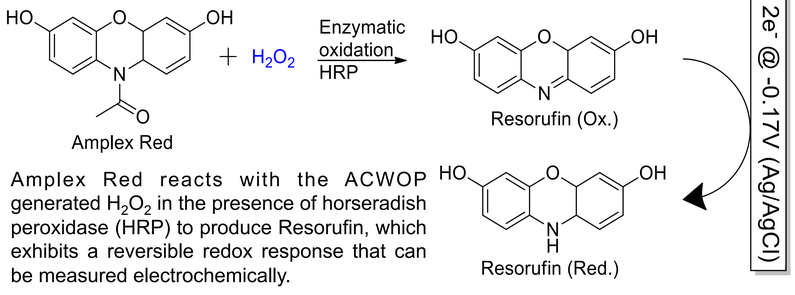
- Labeled secondary reagents are required to bind antibodies, which can reduce signal strength
- Multiple procedural steps can make the technique time intensive

Biosensor Components

As an alternative to ELISA, our biosensor harnesses the intrinsic catalytic activity of all antibodies: the Antibody Catalyzed Water Oxidation Pathway (ACWOP) initially described by Wentworth *et al*. This pathway is independent of specificity, class, and species of antibody and produces up to 500 mole equivalents of hydrogen peroxide (H_2O_2) per antibody, a level of H_2O_2 readily detectable and quantifiable with our biosensor.

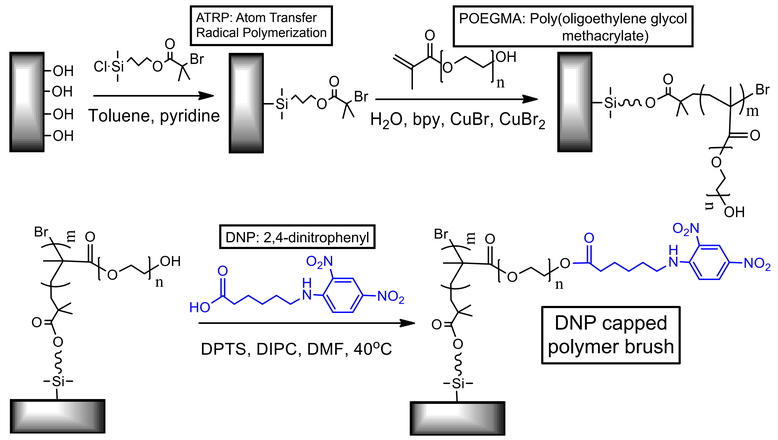


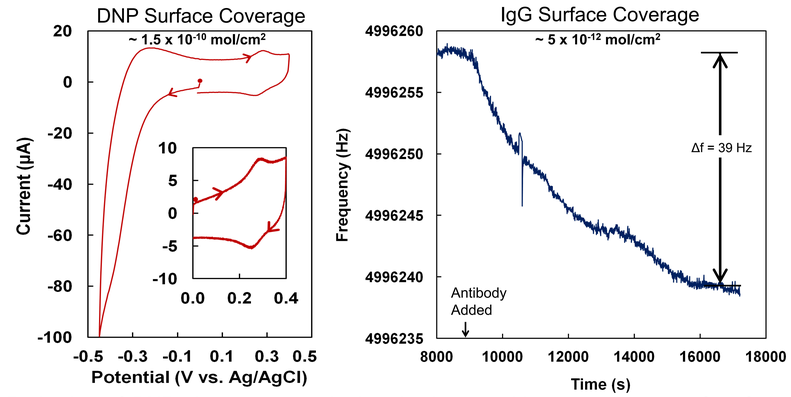
Antibodies are immobilized by 2,4-dinitrophenyl (DNP) molecules attached to polymer brushes. With the help of a photosensitizer and UV light, singlet oxygen ($^1O_2^*$) is generated. Singlet oxygen in the presence of water allows immobilized antibodies to catalyze the production of hydrogen peroxide.



Amplex Red reacts with the ACWOP generated H_2O_2 in the presence of horseradish peroxidase (HRP) to produce Resorufin, which exhibits a reversible redox response that can be measured electrochemically.

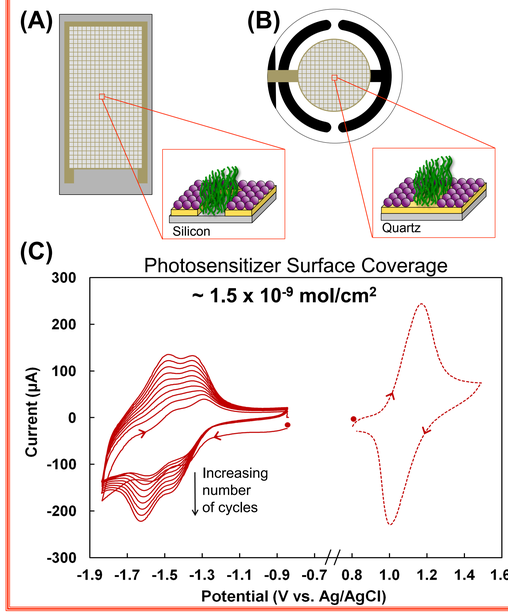
Polymer Brush Synthesis & Surface Characterization





Reduction of DNP nitro groups to hydroxylamines enables measurement of surface coverage by cyclic voltammetry (above left). Quartz crystal microbalance (QCM) measurements determine the surface coverage of IgG antibodies (above right). These results confirm satisfactory capture of desired antibodies on our biosensor surface.

Polymer Brush & Photosensitizer Patterning



Two biosensor platforms present polymer brushes adjacent to the photosensitizer.

(A) Silicon surfaces are used for the ultimate design of a microfluidic device.

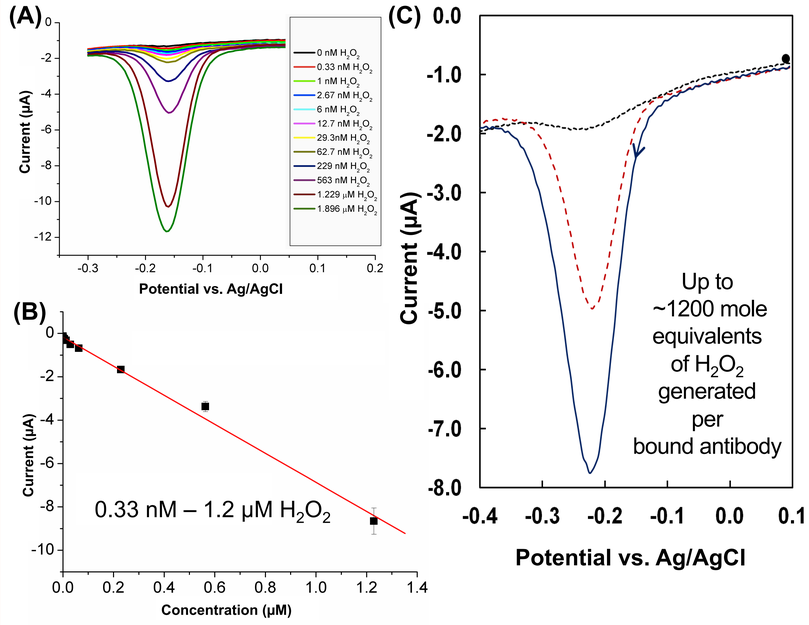
(B) QCM crystals are used for the quantification of bound IgG antibody.

(C) Cyclic voltammogram of QCM shows the electro-polymerization of photo-sensitizer upon reduction (solid lines) and then measured in fresh buffer solution (dashed lines).

Electropolymerized films of photosensitizer directly adjacent to the POEGMA brushes ensures maximum production of H_2O_2.

Hydrogen Peroxide Electrochemistry

The antibody generated H_2O_2 is quantified using square-wave voltammetry (SWV) for high sensitivity at low analyte concentrations on the QCM surface.



(A) SWV of 10 μM Amplex Red and 0.2 units/L horseradish peroxidase; varying concentrations of H_2O_2 are used to construct a calibration curve.

(B) An additional calibration curve indicates the lower limit of H_2O_2 detection is 0.33 nM with an upper limit of 1.2 μM.

(C) SWV shows the detection of H_2O_2 via Resorufin reduction at a 3 mm glassy carbon electrode following irradiation with UV light. Black dashed line is 10 μM Amplex Red; red dashed line is 10 μM Amplex Red with 0.2 units/L HRP before binding antibody; blue solid line is 10 μM Amplex Red with 0.2 units/L HRP in the presence of captured antibody.

Summary

In summary, we have developed a general immunobiosensor platform, employing patterned polymer brushes with photosensitizer films based on the electrochemical detection of H_2O_2 generated through the ACWOP. We have demonstrated the complete function of the biosensor using anti-DNP antibodies as a model system. Moreover, since the ACWOP is a general characteristic of antibodies, our biosensor can, in principle, be applied to virtually any antibody.

We are currently exploring options for integrating this approach with microfluidic platforms and flexible electronics to enable widespread use and field deployment.

REFERENCE

Wentworth, P., Jr, Jones, L.H., Wentworth, A.D., Zhu, X., Larsen, N.A., Wilson, I.A., Xu, X., Goddard, W.A., 3rd, Janda, K.D., Eschenmoser, A., et al. (2001). Antibody catalysis of the oxidation of water. *Science 293*, 1806–1811.

Acknowledgements

IGERT Integrative Graduate Education and Research Traineeship

CNF Cornell NanoScale Science and Technology Facility

NBTC Nanobiotechnology Center