PERSPECTIVE

Color Me Sensitive: Amplification and Discrimination in Photonic Silicon Nanostructures

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ABSTRACT A new paper in this issue discusses some of the considerations for fabrication of biosensor devices in the porous silicon material system. The results focus on optical detection of protease activity on specific substrates that have been immobilized in a porous silicon matrix. Solutions to important limitations of porous microsensors are presented: stabilizing the sensor against corrosion-induced zero point drift, minimizing the effects of nonspecific protein binding, and enhancing the optical response by incorporation of a catalytic reaction in the sensing scheme.

See the accompanying Article by Kilian *et al.* on p 355.

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Electrochemical etching of silicon in HF-containing electrolytes produces porous nanostructures. The size, shape, and population of the pores in a growing film are determined by the current, allowing one to "dial in" a specific porosity pattern in this material. By applying a periodic current–time waveform, porous multilayers that act as one-dimensional photonic crystals can be generated. These dielectric stacks reflect specific wavelengths of light in the visible to near-infrared region of the spectrum. The optical spectrum is sensitive to the refractive index of any molecules filling the pores, and the introduction or removal of a biochemical species can be detected as a shift in wavelength of the characteristic spectral peak. A current trend is to build multistage nanoscale reactors from these materials: controlling the size and shape of the pores to separate molecules, incorporating enzymes to perform a protein-specific catalytic reaction, and harnessing the photonic properties to transduce a reaction into an observable optical response.

SENSITIVITY

To many, sensitivity is thought to be the biggest problem in the sensors field. Indeed, the push to drive detection to a few hundreds of molecules, or even to the single-molecule limit, has produced some heroic results in recent years.[1–3] So how much more sensitive do our sensors need to be? As an esteemed scientist put it at a recent NIH nanomedicine conference, "you can always put on the sunglasses" if a sensor's response is shining too brightly. It definitely helps to have a low limit of detection, but even the best Ray-Bans will not let you see the stars in the middle of the day. The problem of interference often overwhelms a sensor well before it hits its ultimate detection limit. This is particularly true of biosensors; interfering molecules, even those with a relatively weak affinity for the sensor's recognition elements, are usually present at such high concentrations relative to the analyte of interest that they saturate the sensor. So, the key sensor problem is one of discrimination—eliminating the effects of nonspecific interactions from other molecules that can blind your sensor before it has a chance to see its intended target. A general solution is to provide a means of analyte concentration, a mechanism for specifically focusing on the target molecule in preference to everything else in the sample. The standard approach with biosensors is to use a capture probe, such as an antibody, and to bolster the antibody's effectiveness by masking the rest of the sensor surface with a nonsticky coating, typically poly(ethylene glycol) (PEG). Discrimination also has a time component to it. Thermal or chemical fluctuations in the

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sample matrix can cause a sensor to drift, giving a different reading on a different day or even from minute to minute. Ideally, the operational mechanism of the sensor must provide a low detection limit with high discrimination and no drift.

The discrimination problem is a particular challenge for portable sensors. A sensor used outside of the clinical or analytical laboratory setting does not have the luxury of near-infinite supplies of reagents, power, and space. In addition, the requirements of device engineering and rapid turnaround time limit the amount of sample cleanup that can be performed prior to analysis. The promise of nanotechnology is that it can allow us to design some of the key sample preparation, processing, and signal conversion steps directly into the sensor element. In their article this issue, Gooding and co-workers demonstrate some of these functions in a nanostructured porous silicon-based biosensor, using the detection of protease activity as an exemplar.[4]

PROGRAMMABLE NANOSTRUCTURES

Silicon displays a peculiar electrochemical behavior in electrolytes containing hydrofluoric acid (HF). When an anodic current is imposed, nanometer-scale pores drill into the wafer, removing silicon in the form of the hexafluorosilicate ion (SiF_6^{2-}). The nanostructure that remains is a high surface area form of silicon which retains the crystallinity of the silicon wafer from which it was produced. The average pore size can be controlled over a wide range by appropriate choice of current, HF concentration, wafer resistivity, and electrode configuration. Probably the most amazing aspect of this electrochemical system is that the porosity of a growing layer tracks the electrochemical current applied during the etch.[5] A cross-sectional scanning electron microscope image demonstrating this feature is shown in Figure 1. For that sample, the current was suddenly decreased approximately halfway through the etch, resulting in the abrupt decrease in pore size shown. The tunability of the pore etching process provides a convenient means to build nanostructured matrices that can act as reactors,[6] reservoirs,[7,8] or size-exclusion membranes.[9,10] The programmability can also be used to build optical structures with very interesting properties.

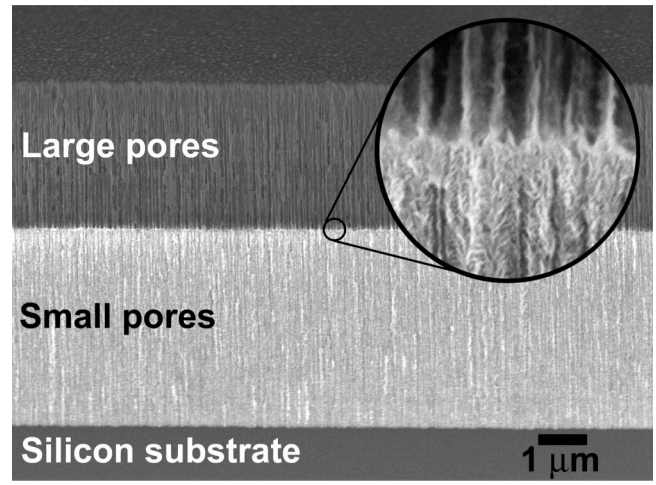

Figure 1. Designer pores. Cross-sectional electron microscope image of a porous silicon sample containing two distinct pore morphologies. The morphology is controlled by the current applied during etching. In this sample, the current was decreased suddenly during preparation, resulting in the abrupt decrease in pore diameter observed. Sample courtesy Manuel Orosco, University of California, San Diego. Electron micrograph courtesy Melanie L. Oakes, Hitachi Chemical Research Center, Irvine, CA. Inset is 590 nm in diameter.

The ability to program the nanostructure of a porous silicon film with a current–time etching waveform allows the construction of photonic materials with precisely defined spectral features. Vincent[11] was the first to recognize the potential of this method for the preparation of one-dimensional photonic crystals; Berger[12,13] and many others quickly elaborated. Multilayers are prepared by periodically varying the current density during the etching process: a current *versus* time profile can be transferred to a porosity (*i.e.*, refractive index) *versus* depth profile. The beautifully stratified structure shown in Figure 1A of the Gooding *et al.* paper was prepared this way, using a sinusoidal current modulation.[4]

The wavelength maximum of a one-dimensional photonic crystal is determined by the period of the refractive index gradient in the film, and so it is very easy to "program" a specific spectrum into a porous silicon chip with the etching power supply.[14,15] The optical spectrum of one of these samples appears to the naked eye as a distinct color (Figure 2), and sharp spectral features can be engineered to appear anywhere in the visible to near-infrared region of the spectrum, allowing great flexibility in the design of optical transducers used in molecular sensing. The work described in the Gooding paper uses a fairly expensive spectrograph, but low-cost CCD spectrometers,[16,17] diode laser interferometers,[18] and LED/phototransistor systems[19] have also been used to measure the spectral changes needed for sensor operation. Sensing of fairly low concentrations of chemical or biochemical species can even be performed by the naked eye with this type of optical structure.[20,21]

BETTER SENSING THROUGH CHEMISTRY

The wavelength of the spectral peak reflected from the porous silicon photonic crystal is dependent on the refractive index of the porous matrix. Gooding and co-workers attach proteins to the inner pore walls of their film and, through the action of a protease, are able to clip fragments of the protein from the surface very specifically. Changes in refractive index of the porous layer as the protein fragments diffuse away results in a blue shift of the reflectivity peak,

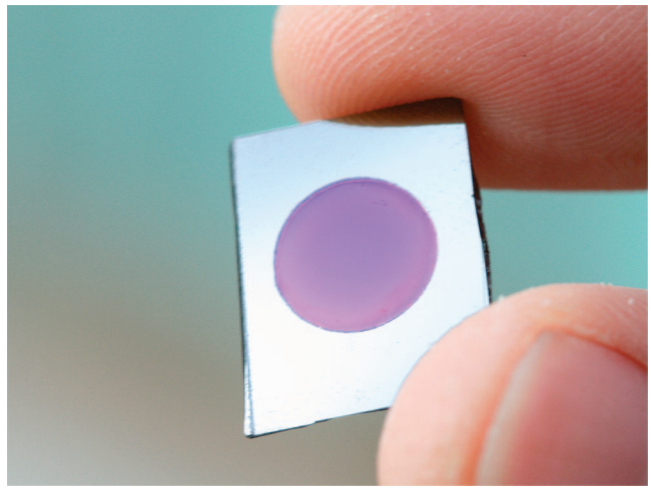

Figure 2. Structural color from a porous silicon multilayer. This particular type of photonic crystal is called a rugate filter; it possesses a sinusoidal porosity gradient in the *z*-direction. The color derives from a modulation in refractive index, determined by the wavelength of the current–time waveform used to prepare the chip. Photo courtesy Luo Gu, University of California, San Diego.

producing an observable color change. By exploiting the specific catalytic activity of the protease, Gooding and co-workers are able to achieve a detection limit of 37 nM, corresponding to a few hundred nanograms of protease.

The use of an enzymatic reaction to increase the sensitivity of an assay is a powerful approach, with many successful examples in biotechnology (*e.g.*, the ELISA assay, the polymerase chain reaction, *etc.*). The coupling of enzymatic (and more generally, catalytic) reactions to assays is a growing trend in the nanosensors community, and many nanosystems are amenable to catalytic amplification.[21–27] Silicon-based catalytic sensors face two challenges: the host matrix cannot inhibit the catalytic reaction,[27] and the catalytic reaction or its byproducts cannot destroy the host.

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RUST NEVER SLEEPS

Silicon is thermodynamically unstable, oxidizing to SiO_2 in air or water. With its high surface area, porous Si is particularly susceptible. Once oxidized, nanophase SiO_2 readily dissolves in aqueous media,[28] and surfactants or nucleophiles accelerate the process.[29,30] Both the oxidation and the dissolution processes alter the refractive index of a porous Si film, producing unacceptable drift in the optical response of the sensor. Gooding and co-workers solve the stability problem by chemically grafting an organic species directly to the surface via Si-C bonds.

There are two main methods for attaching organic molecules to Si surfaces: the first involves Si−O bonds and the second involves Si−C bonds. While Si−O bonds are easy to prepare (by oxidation) and functionalize (by silanol chemistry), they are susceptible to nucleophilic attack, and thus they are not particularly stable in biological media. First recognized by Chidsey and co-workers at Stanford University,[31] the lower electronegativity of carbon relative to oxygen translates to greater kinetic stability of Si−C grafted species on silicon surfaces. Gooding and co-workers form Si−C bonds to their porous Si surface using a hydrosilylation reaction that was popularized by Buriak[32,33] and elaborated on by Boukherroub, Chazalviel, Lockwood, and others.[34–37]

Thermal hydrosilylation is the "alkanethiols on gold" analogue reaction of the silicon system, allowing the chemist to place a wide variety of organic functional groups on a silicon or porous silicon surface.[32] The main requirement of the reaction is that the silicon surface contain Si-H species so they can react with a terminal alkene (or alkyne) on the organic fragment; porous Si comes out of the etching bath covered with these hydrides.

The hydrosilylation reaction stabilizes the porous Si sensor in aqueous media, but the surface must still avoid the nonspecific binding of interfering proteins and other species that can mask the sensor from its target. The reaction employed by Gooding and co-workers[38] follows on a published method to place a PEG linker on a

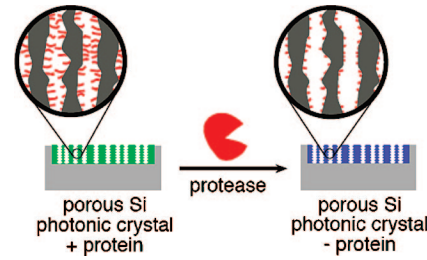

Figure 3. Catalytic amplification applied to an optical sensor. A porous Si photonic crystal is modified with a protein substrate. Exposure to a protease cleaves the protein from the surface, shifting the color of the photonic crystal to the blue. The protein is tethered to the surface *via* Si−C bonds, inhibiting corrosion of the porous nanostructure. The chemistry also includes a poly(ethylene glycol) (PEG) linker between the protein and the surface bond to minimize nonspecific binding. Both the Si−C and the PEG chemistries suppress zero-point drift and stabilize the sensor response.

porous Si surface.[39] The short-chain PEG linker yields a hydrophilic surface that is capable of admitting biomolecules into the pores without binding them strongly. The distal end of the PEG linker is modified to allow protein coupling, and the proteins used as "bait" for the target protease are then attached (Figure 3).

LIMITATIONS AND FUTURE DIRECTIONS

The high surface area afforded by a porous material provides greater sensitivity because it accommodates more target or capture probes. However, such structures can be fragile and unstable, and if the pores are too small they can inhibit the binding or catalytic processes they are intended to sense. The work presented by Gooding and co-workers solves several key problems in label-free sensing with porous photonic materials. They provide a chemical reaction to stabilize the surface and inhibit nonspecific binding, a catalytic reaction to amplify the response, a matrix to contain the reaction, and a means to engineer an optical spectrum to allow detection of the result. These and other results show that nanotechnology allows us to design much more than sensitivity into the sensor element.

REFERENCES AND NOTES

1. Moerner, W. E. New Directions in Single-Molecule Imaging and Analysis. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 12596–12602.
2. Michaels, A. M.; Nirmal, M.; Brus, L. E. Surface Enhanced Raman Spectroscopy of Individual Rhodamine 6G Molecules on Large Ag Nanocrystals. *J. Am. Chem. Soc.* **1999**, *121*, 9932–9939.
3. Elghanian, R.; Storhoff, J. J.; Mucic, R. C.; Letsinger, R. L.; Mirkin, C. A. Selective Colorimetric Detection of Polynucleotides Based on the Distance-Dependent Optical Properties of Gold Nanoparticles. *Science* **1997**, *277*, 1078–1081.
4. Kilian, K. A.; Böcking, T.; Gaus, K.; Gal, M.; Gooding, J. J. Peptide-Modified Optical Filters for Detecting Protease Activity. *ACS Nano* **2007**, *4*, 355–361.
5. Zhang, X. G. Morphology and Formation Mechanisms of Porous Silicon. *J. Electrochem. Soc.* **2004**, *151*, C69–C80.
6. Chin, V. I.; Bhatia, S. N.; Sailor, M. J.; Collins, B. E. Nanoporous Silicon Support Containing Macropores for Use as a Bioreactor. U.S. Patent 6,734,000, 2004.
7. Leong, W. Y.; Loni, A.; Canham, L. T. Electrically Enhanced Erosion of Porous Si Material in Electrolyte by pH Modulation and Its Application in Chronotherapy. *Phys. Status Solidi A—Appl. Mater.* **2007**, *204*, 1486–1490.
8. Thomas, J. C.; Pacholski, C.; Sailor, M. J. Delivery of Nanogram Payloads Using Magnetic Porous Silicon Microcarriers. *Lab Chip* **2006**, *6*, 782–787.
9. Striemer, C. C.; Gaborski, T. R.; McGrath, J. L.; Fauchet, P. M. Charge- and Size-Based Separation of Macromolecules Using Ultrathin Silicon Membranes. *Nature* **2007**, *445*, 749–753.
10. Collins, B. E.; Dancil, K.-P.; Abbi, G.; Sailor, M. J. Determining Protein Size Using an Electrochemically Machined Pore Gradient in Silicon. *Adv. Funct. Mater.* **2002**, *12*, 187–191.
11. Vincent, G. Optical Properties of Porous Silicon Superlattices. *Appl. Phys. Lett.* **1994**, *64*, 2367–2369.
12. Berger, M. G.; Dieker, C.; Thonissen, M.; Vescan, L.; Luth, H.; Munder, H.; Theiss, W.; Wernke, M.; Grosse, P. Porosity Superlattices—a New Class of Si Heterostructures. *J. Phys. D—Appl. Phys.* **1994**, *27*, 1333–1336.
13. Loni, A.; Canham, L. T.; Berger, M. G.; Arens-Fischer, R.; Munder, H.; Luth, H.; Arrand, H. F.; Benson, T. M. Porous Silicon Multilayer Optical Waveguides. *Thin Solid Films* **1996**, *276*, 143–146.
14. Berger, M. G.; Arens-Fischer, R.; Thoenissen, M.; Krueger, M.; Billat, S.; Lueth, H.; Hilbrich, S.; Theiss, W.; Grosse, P. Dielectric Filters Made of Porous Silicon: Advanced Performance by Oxidation and New Layer Structures. *Thin Solid Films* **1997**, *297*, 237–240.
15. Meade, S. O.; Yoon, M. S.; Ahn, K. H.; Sailor, M. J. Porous Silicon Photonic Crystals as Encoded Microcarriers. *Adv. Mater.* **2004**, *16*, 1811–1814.
16. Lin, V. S.-Y.; Motesharei, K.; Dancil, K. S.; Sailor, M. J.; Ghadiri, M. R. A Porous Silicon-Based Optical Interferometric Biosensor. *Science* **1997**, *278*, 840–843.
17. Chan, S.; Horner, S. R.; Miller, B. L.; Fauchet, P. M. Identification of Gram Negative Bacteria Using Nanoscale Silicon Microcavities. *J. Am. Chem. Soc.* **2001**, *123*, 11797–11798.
18. Gao, J.; Gao, T.; Sailor, M. J. A Porous Silicon Vapor Sensor Based on Laser Interferometry. *Appl. Phys. Lett.* **2000**, *77*, 901–390.
19. Dorvee, J.; Sailor, M. J. A Low-Power Sensor for Volatile Organic Compounds Based on Porous Silicon Photonic Crystals. *Phys. Status Solidi A—Appl. Mater.* **2005**, *202*, 1619–1623.
20. Sailor, M. J.; Link, J. R. "Smart Dust": Nanostructured Devices in a Grain of Sand. *Chem. Commun.* **2005**, *11*, 1375–1383.
21. Orosco, M. M.; Pacholski, C.; Miskelly, G. M.; Sailor, M. J. Protein-Coated Porous Silicon Photonic Crystals for Amplified Optical Detection of Protease Activity. *Adv. Mater.* **2006**, *18*, 1393–1396.
22. Xu, X. Y.; Georganopoulou, D. G.; Hill, H. D.; Mirkin, C. A. Homogeneous Detection of Nucleic Acids Based upon the Light Scattering Properties of Silver-Coated Nanoparticle Probes. *Anal. Chem.* **2007**, *79*, 6650–6654.
23. Sohn, H.; Létant, S.; Sailor, M. J.; Trogler, W. C. Detection of Fluorophosphonate Chemical Warfare Agents by Catalytic Hydrolysis with a Porous Silicon Interferometer. *J. Am. Chem. Soc.* **2000**, *122*, 5399–5400.
24. Baranzahi, A.; Tobias, P.; Spetz, A. L.; Martensson, P.; Ekedahl, L.-G.; Lundstrom, I. Chemical Sensors with Catalytic Metal Gates. *J. Electrochem. Soc.* **1998**, *145*, 3401–3406.
25. DeLouise, L. A.; Miller, B. L. Enzyme Immobilization in Porous Silicon: Quantitative Analysis of the Kinetic Parameters for Glutathione-S-Transferases. *Anal. Chem.* **2005**, *77*, 1950–1956.
26. Mulchandani, P.; Mulchandani, A.; Kaneva, I.; Chen, W. Biosensor for Direct Determination of Organophosphate Nerve Agents. I. Potentiometric Enzyme Electrode. *Biosens. Bioelectron.* **1999**, *14*, 77–85.
27. DeLouise, L. A.; Miller, B. L. Quantatitive Assessment of Enzyme Immobilization Capacity in Porous Silicon. *Anal. Chem.* **2004**, *76*, 6915–6920.
28. Dancil, K.-P. S.; Greiner, D. P.; Sailor, M. J. A Porous Silicon Optical Biosensor: Detection of Reversible Binding of IgG to a Protein A-Modified Surface. *J. Am. Chem. Soc.* **1999**, *121*, 7925–7930.
29. Bjorklund, R. B.; Zangooie, S.; Arwin, H. Adsorption of Surfactants in Porous Silicon Films. *Langmuir* **1997**, *13*, 1440–1445.
30. Canaria, C. A.; Huang, M.; Cho, Y.; Heinrich, J. L.; Lee, L. I.; Shane, M. J.; Smith, R. C.; Sailor, M. J.; Miskelly, G. M. The Effect of Surfactants on the Reactivity and Photophysics of Luminescent Nanocrystalline Porous Silicon. *Adv. Funct. Mater.* **2002**, *12*, 495–500.
31. Linford, M. R.; Chidsey, C. E. D. Alkyl Monolayers Covalently Attached to

Silicon Surfaces. *J. Am. Chem. Soc.* **1993**, *115*, 12631–12632.
32. Buriak, J. M. Organometallic Chemistry on Silicon and Germanium Surfaces. *Chem. Rev.* **2002**, *102*, 1272–1308.
33. Buriak, J. M.; Allen, M. J. Lewis Acid Mediated Functionalization of Porous Silicon with Substituted Alkenes and Alkynes. *J. Am. Chem. Soc.* **1998**, *120*, 1339–1340.
34. Boukherroub, R.; Petit, A.; Loupy, A.; Chazalviel, J. N.; Ozanam, F. Microwave-Assisted Chemical Functionalization of Hydrogen-Terminated Porous Silicon Surfaces. *J. Phys. Chem. B* **2003**, *107*, 13459–13462.
35. Boukherroub, R.; Wojtyk, J. T. C.; Wayner, D. D. M.; Lockwood, D. J. Thermal hydrosilylation of undecylenic acid with porous silicon. *J. Electrochem. Soc.* **2002**, *149*, 59–63.
36. Mattei, G.; Valentini, V. *In Situ* Functionalization of Porous Silicon During the Electrochemical Formation Process in Ethanoic Hydrofluoric Acid Solution. *J. Am. Chem. Soc.* **2003**, *125*, 9608–9609.
37. Boukherroub, R.; Wojtyk, J. T. C.; Wayner, D. D. M.; Lockwoodb, D. J. Thermal Hydrosilylation of Undecylenic Acid with Porous Silicon. *J. Electrochem. Soc.* **2002**, *149*, H59–H63.
38. Kilian, K. A.; Bocking, T.; Gaus, K.; Gal, M.; Gooding, J. J. Si-C Linked Oligo(ethylene glycol) Layers in Silicon-Based Photonic Crystals: Optimization for Implantable Optical Materials. *Biomaterials* **2007**, *28*, 3055–3062.
39. Schwartz, M. P.; Cunin, F.; Cheung, R. W.; Sailor, M. J. Chemical Modification of Silicon Surfaces for Biological Applications. *Phys. Status Solidi A—Appl. Mater.* **2005**, *202*, 1380–1384.