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A General Photonic Crystal Sensing Motif: Creatinine in Bodily Fluids

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Abstract: We developed a new sensing motif for the detection and quantification of creatinine, which is an important small molecule marker of renal dysfunction. This novel sensor motif is based on our intelligent polymerized crystalline colloidal array (IPCCA) materials, in which a three-dimensional crystalline colloidal array (CCA) of monodisperse, highly charged polystyrene latex particles are polymerized within lightly cross-linked polyacrylamide hydrogels. These composite hydrogels are photonic crystals in which the embedded CCA diffracts visible light and appears intensely colored. Volume phase transitions of the hydrogel cause changes in the CCA lattice spacings which change the diffracted wavelength of light. We functionalized the hydrogel with two coupled recognition modules, a creatinine deiminase (CD) enzyme and a 2-nitrophenol (2NPh) titrating group. Creatinine within the gel is rapidly hydrolyzed by the CD enzyme in a reaction which releases OH^-. This elevates the steady-state pH within the hydrogel as compared to the exterior solution. In response, the 2NPh is deprotonated. The increased solubility of the phenolate species as compared to that of the neutral phenols causes a hydrogel swelling which red-shifts the IPCCA diffraction. This photonic crystal IPCCA senses physiologically relevant creatinine levels, with a detection limit of 6 μM, at physiological pH and salinity. This sensor also determines physiological levels of creatinine in human blood serum samples. This sensing technology platform is quite general. It may be used to fabricate photonic crystal sensors for any species for which there exists an enzyme which catalyzes it to release H^+ or OH^-.

Introduction

There is an ever increasing demand for simple, selective, accurate, and reliable assay methods for determining clinically important metabolites as indicators of health status.[1] For example, creatinine is an important diagnostic substance for muscle mass and renal function.[1] Creatinine levels in bodily fluids such as serum or plasma are typically around 35−140 μM in healthy adults.[2] However, creatinine levels in the serum or plasma of patients with renal dysfunction may be as high as 1 mM.[3] The conventional methods used to quantify creatinine levels in bodily fluids involve either colorimetric analysis using the Jaffe reaction[4] or an enzyme cascade reaction which involves converting creatinine to release H_2O_2 in four enzymatically catalyzed, coupled reaction steps, followed by the spectrophotometric quantification of the H_2O_2 produced with leuco dye and peroxidase.[5] Both of these techniques can be fully automated to analyze creatinine levels in bodily fluids.[1,2,5] However, the Jaffe reaction for creatinine quantification is not selective for creatinine alone. Other species present in the bodily fluid such as sugars, urea, uric acid, pyruvate, and dopamine can interfere severely with this technique.[4] The enzyme cascade technique is highly selective for creatinine; however, the high cost of preparation of the enzyme cascade film element may limit the utility of this technique.[5] In addition, neither of these techniques has the potential to be used for continuous, in-vivo, creatinine quantification.

To address problems with these two widely used creatinine assay techniques, researchers are investigating alternative approaches. One alternate utilizes small molecule hydrogen bonding receptors which selectively bind creatinine.[6] The creatinine concentration would be quantified spectrophotometrically. While attractive, this technique may not be sufficiently sensitive for the creatinine levels found in bodily fluids due to the weak nature of the hydrogen bonding host−guest complexation involved. Other approaches include amperometric mono-enzyme and trienzyme cascade biosensors,[7−14] potentiometric

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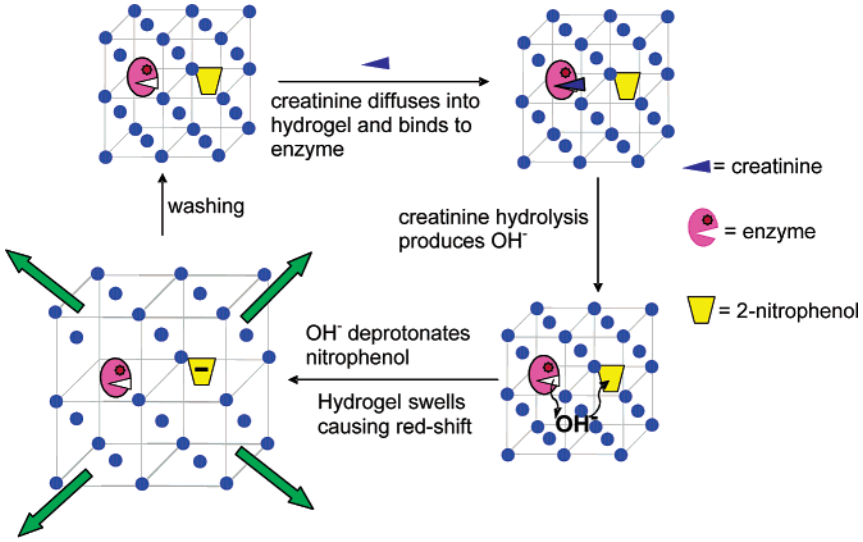


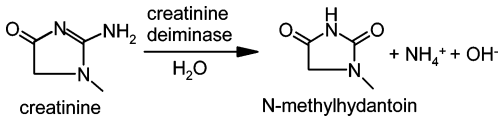
Figure 1. Schematic depiction of the IPCCA sensor concept.

ion sensitive field effect transistor (ISFET) creatinine sensors coupled to ion or gas sensitive electrodes,[15−19] impedimetric creatinine sensors,[20,21] and an optical creatinine sensor.[22] Almost all of these approaches have problems such as low selectivity, low sensitivity, and reproducibility limitation for creatinine assays.

Recently, a very sensitive, selective, and stable creatinine biosensor was demonstrated which utilized a differential pair of ISFETs with creatinine deiminase (CD) enzyme immobilized on the transducer surface.[1] This system detects the change in pH when creatinine is selectively hydrolyzed by the enzyme. This system is one of the most promising creatinine quantification techniques reported thus far, in that the sensor device may be easily miniaturized, cheaply mass produced, and can be used to (ex-situ) quantify creatinine levels in bodily fluid samples.[1]

There are thousands of patients with severe renal dysfunction who depend on routine dialysis.[2] A reliable, inexpensive, and visual (ex-situ or in-vivo implantable) bodily fluid creatinine sensor, which does not require expensive detection instrumentation or draw electrical power, would be invaluable to such patients.[1,2,21] Such a sensing motif would be ideal for patients with renal disease to accurately monitor their own blood creatinine levels at home. Patients, who have undergone kidney transplantation, may be able to use such a device to determine if the newly implanted kidney is undergoing rejection by their immune systems. These potential applications may lead to significant cost savings for patients and healthcare providers alike.

Scheme 1. Hydrolysis of Creatinine Using Creatinine Deiminase Raises the pH of the Reaction Medium



In this publication, we describe a novel sensing motif based on our intelligent polymerized crystalline colloidal array (IPCCA) technology[23−27] for determination of creatinine in bodily fluids. We already demonstrated that our IPCCA technology can be utilized to prepare chemical sensors for the visual determination of various analytes such as glucose[24−26] and metal cations.[27] The IPCCA sensing technology utilizes a hydrogel which contains an fcc array of colloidal particles that diffracts light in the visible spectral region (Figure 1). The volume of this hydrogel and the wavelength of light it diffracts are a function of analyte concentration. Thus, the analyte concentration is determined from the wavelength of light diffracted by the IPCCA sensor. These sensing materials are inexpensive, are easy to miniaturize, and require no instrumentation to operate.[23−27]

The molecular recognition agent in our sensor is creatinine deiminase (CD), an enzyme which rapidly hydrolyzes creatinine to *N*-methylhydantoin (Scheme 1), with release of OH^-.[1,28]

This hydroxide production increases the steady-state pH within the PCCA hydrogel over that outside. We then couple a second recognition module, 2-nitrophenol, which titrates in response to the resulting pH changes. Deprotonation of the phenol group causes the PCCA hydrogel to swell due to the

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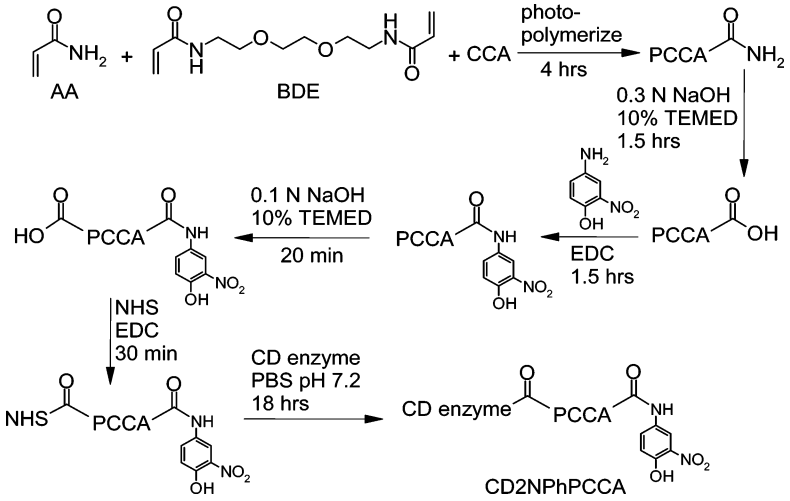
Scheme 2. Preparation of Creatinine PCCA Sensor



increased solubility of the phenolate. The net result is that our sensor increasingly red-shifts its diffraction, as the creatinine concentration in solution increases (Figure 1).

Experimental Section

All diffraction measurements were performed in reflectance mode with an Ocean Optics OOI base 32 spectrometer. UV−vis transmission spectra were measured by using a Perkin-Elmer lambda 9 UV/vis/NIR spectrophotometer, operating in single beam mode.

4-Acetamido-2-nitrophenol was prepared in a manner similar to that described by Hutton et al.[29] *N*,*N*′-Bisacryloyl diamino ethoxyethane (BDE) was prepared in a manner similar to that described by Stahl et al.[30]

Polymerization of PCCA. The preparation of the PCCA is depicted in Scheme 2. A polymerization mixture consisting of acrylamide (AA, 0.100 g, 1.40 mmol, Bio-Rad), BDE (0.005 g, 0.01951 mmol), CCA (2.00 g, 8 wt %, 120 nm diameter polystyrene latex spheres), and AG 501-X8 ion-exchange resin (0.10 g, 20−50 mesh, mixed bed, Bio-Rad) was prepared. 10% w/w diethoxyacetophenone (DEAP 7.7 μL, 3.84 μmol, Aldrich) in dimethyl sulfoxide (DMSO, Fisher) was added to this mixture, and the mixture was taken up through a thin needle to filter out the ion-exchange resin. The mixture was injected into a polymerization cell consisting of two quartz disks, separated by a ∼125 μm thick Parafilm spacer. The polymerization cell was placed between two mercury (Black Ray) lamps operating at 365 nm for 4 h. The cell was opened in Nanopure water, and the PCCA was washed thoroughly with Nanopure water (λ_{max} = 530 nm in water). The polystyrene colloids were prepared as described elsewhere.[31]

Hydrolysis of PCCA. The PCCA (1.5 cm × 1.5 cm × 125 μm) was placed in 25 mL of an aqueous hydrolysis solution containing 0.3 N NaOH (Fisher) with 10% v/v *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED, Aldrich) for 1.5 h. The hydrolyzed PCCA was washed thoroughly with 150 mM NaCl (Fisher) solution.

Preparation of pH Sensitive PCCA. 4-Amino-2-nitrophenol (0.130 g, 0.843 mmol, Acros) was weighed into a 2 dram sample vial and was dissolved completely in 4 mL of DMSO. The DMSO solution was placed in a 60 mL square plastic media bottle and was diluted to 50 mL with Nanopure water. The pH was adjusted to 4.0 with concentrated HCl (Fisher), and then solid ethyl-3-(3-dimethylamino-propyl) carbodiimide HCl (EDC, 0.200 g, 1.04 mmol, Pierce) was dissolved in this solution. This solution was added to the hydrolyzed PCCA, and the coupling reaction was allowed to proceed over 1.5 h at room temperature. The resulting PCCA with appended 2-nitrophenol groups was washed thoroughly with Nanopure water (the diffraction maximum was found to occur at λ_{max} = 480 nm in water). This PCCA was also used to study the effect of pH on phenol deprotonation and to characterize the hydrogel swelling as a function of ionic strength.

Second Hydrolysis of PCCA. The pH sensitive PCCA was placed in 25 mL of an aqueous solution containing 0.1 N NaOH and 10% v/v TEMED for 20 min. The resulting hydrolyzed PCCA was washed thoroughly in 150 mM NaCl (λ_{max} = 470 nm in 150 mM NaCl).

Preparation of Reactive PCCA. *N*-Hydroxysuccinamide (NHS, 0.140 g, 1.22 mmol, Aldrich) was dissolved in 50 mL of 0.1 M morpholinoethane sulfonate (MES, Aldrich) buffered saline at pH 6.0. Solid EDC (0.180 g, 0.936 mmol) was dissolved in this solution. The hydrolyzed PCCA was placed in this solution for 30 min, and the resulting PCCA was washed thoroughly with standard phosphate buffered saline (PBS, Aldrich) at pH 7.2. Creatinine deiminase enzyme (CD) was immediately attached to this hydrogel.

Preparation of Creatinine IPCCA Sensor. The solution containing CD (microbial, 0.0035 g, MW 288 KDa, Sigma) in 0.7 mL of PBS buffer at pH 7.2 was placed onto the PCCA in a Petri dish, and the system was kept in the dark at room temperature for 18 h. The resulting creatinine IPCCA sensor was washed thoroughly with PBS solution and was stored in PBS at pH 7.2 in a refrigerator at 4 °C (λ_{max} = 460 nm in PBS at pH 7.2).

Preparation of CCA Free Hydrogels. CCA free hydrogels for the spectral determination of enzyme and phenol concentrations were prepared using identical techniques, except pure water was added instead of CCA solution.

Results and Discussion

Our goal is to develop a sensing technology which operates at the high ionic strengths and the physiological pH values of bodily fluids. An ultimate goal is to use bodily fluids directly without sample preparation. We ultimately wish to use, for example, blood sampled by finger prick or urine directly. We also desire to have an optical color readout of the analyte level which can be visually determined by matching the color observed with a color chart.

To accomplish this, we coupled the selectivity of an enzyme reaction which liberates OH^- to a pH responsive PCCA. We earlier demonstrated[32] a pH responsive hydrogel for low ionic

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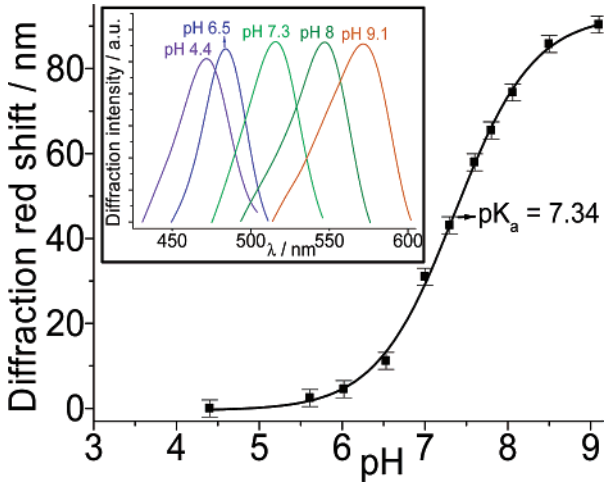

Figure 2. pH response of 2-nitrophenol PCCA in aqueous solution containing 150 mM NaCl. The inset shows the changes in the diffraction maximum and visually perceived color of the PCCA as a function of pH.

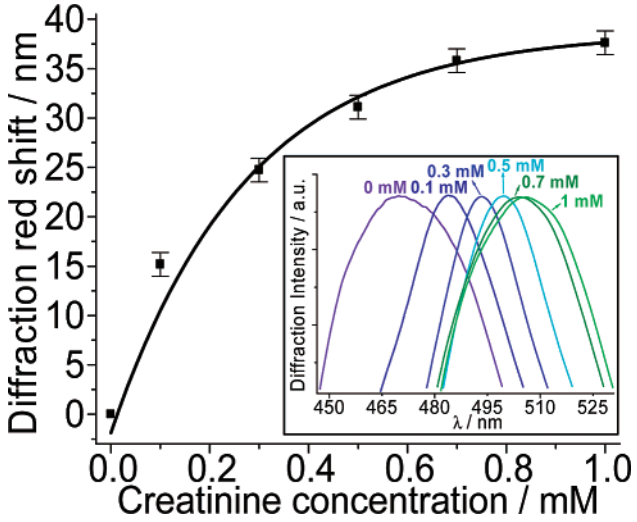

Figure 3. Creatinine concentration dependence of diffraction from our PCCA creatinine sensor in 5 mM Tris (pH 7.4) in the presence of 150 mM NaCl. The solid line is a theoretical fit to the response from the model described below.

strength aqueous solutions, whereby deprotonation of titrable groups formed an ionic hydrogel. The resulting Donnan potential caused hydrogel swelling. We are unable to use this motif in bodily fluids due to their large ionic strength. No swelling can occur due to a Donnan potential for ionic strengths greater than ~10 mM equivalent in NaCl, for example.[24,32]

Instead, we utilized a 2-nitrophenol PCCA which should be pH responsive; the free energy of mixing of phenolate should be greater than that of phenol. This should result in PCCA swelling at high pH.[33]

We examined the dependence of a 2-nitrophenol PCCA upon pH in the presence of 150 mM NaCl. The IPCCA swells as the solution ionic strength increases due to the higher solubility of the polyacrylamide network in salt solutions. Hence, we preequilibrate the gel in a pH 7.4 buffer solution with 150 mM NaCl, close to that found in bodily fluids. The diffraction red-shifts from ~470 (blue) to ~589 nm (yellow) between pH = 4.4 and pH = 9.1 with an apparent pK_a of 7.34[34] as shown in Figure 2. The response is completely reversible; a pH decrease shrinks the hydrogel back to its original diffraction wavelength. We do not expect phenols to bind to metal ions at the concentrations normally found in bodily fluids.[35] This Figure 2 hydrogel reversible volume swelling upon pH increase results from a more favorable free energy of mixing of the phenolate derivative than for the phenol.

Creatinine Concentration Dependence of PCCA Diffraction. Our IPCCA creatinine sensor contains both 2-nitrophenol and CD, which metabolizes creatinine and releases hydroxide (Scheme 1). This will increase the internal IPCCA pH, which will swell the IPCCA and red-shift the diffraction in proportion to the creatinine concentration in the exterior solution. Figure 3 shows the creatinine concentration dependence of our PCCA sensor diffraction in a 5 mM Tris−HCl buffer at pH 7.4 in the presence of 150 mM NaCl. The diffraction monotonically red-shifts with increasing creatinine concentrations and begins to saturate at a ~1 mM creatinine concentration. The sensing response is fully reversible and reproducible. This range of creatinine concentrations determined is similar to that in human blood and plasma; 40−120 μM concentrations occur in normal people, while up to 900 μM concentrations are found in patients with severe renal failure.[2,3] The solid line in Figure 3 is the response calculated from the model described below. The theoretical calculation fits the experimentally observed response well.

The reproducibility in reading the diffraction red-shift appears to be the major error in the measurement. The magnitude of this error, 3σ ~0.6 nm, allows us to estimate a creatinine detection limit of ~6 μM under these sensing conditions. This detection limit is comparable to that of the existing creatinine measurement techniques.[1]

An IPCCA which contains only 2-nitrophenol groups or only the CD enzyme does not respond to creatinine under the above conditions. Hence, both species are necessary for the PCCA response.

Dependence of Creatinine Sensing on Buffer Concentration. The steady-state pH difference between the interior of the PCCA sensor and the exterior analyte solution should decrease as the buffer concentration increases; in the limit of infinite buffer concentration, no pH difference could occur. Figure 4 shows the buffer concentration dependence of diffraction of our sensor in a 0.5 mM creatinine, 150 mM NaCl solution. The magnitude of the diffraction red-shift in response to creatinine decreases dramatically as the buffer concentration increases. The red-shift becomes negligible by 50 mM buffer. The solid line in Figure 4 shows that our model reasonably fits the response dependence on buffer concentration.

Measurement of Creatinine in Human Serum Samples. We determined the creatinine concentrations in discarded anonymous human serum samples obtained from the University of Pittsburgh Medical Center Clinical Laboratories.

We initially measured diffraction from a fresh IPCCA sensor in 20 mM Tris−HCl buffer containing 150 mM NaCl at pH 7.4. We then drained the solution and covered the IPCCA with 0.4 mL of serum having a creatinine concentration of ~0.01 mM. Serum contains proteins which diffuse into the IPCCA

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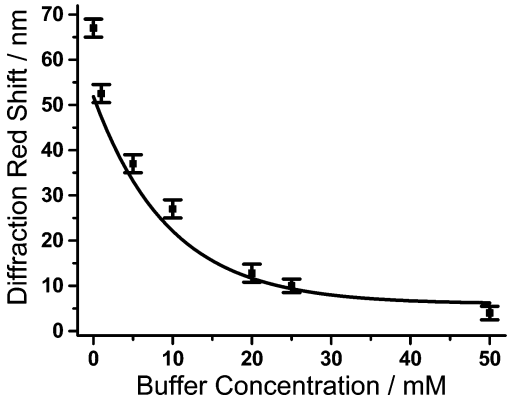

Figure 4. Buffer concentration dependence of the diffraction red-shift response of PCCA sensor to 0.5 mM creatinine concentration in 150 mM NaCl at pH 7.4. The solid line is a theoretical fit to the data.

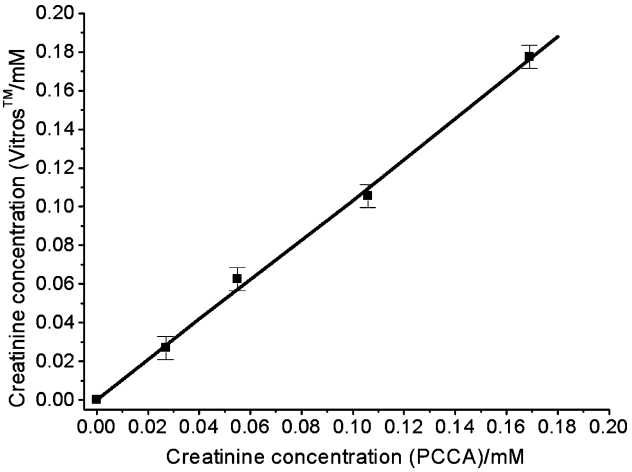

Figure 5. Comparison between human serum creatinine concentration determined by the Vitros autoanalyzer and by using the IPCCA creatinine sensor.

sensor and blue-shift the diffraction. After the blue-shift saturated, we removed the serum buffer and covered the IPCCA with 0.4 mL of serum with a higher creatinine concentration. The IPCCA volume was 0.01 mL, while that of the serum used to cover the PCCA was 0.4 mL. We quantified the creatinine concentration by measuring the diffraction red-shift after it saturated. The serum was removed, and then next serum sample was placed on the IPCCA sensing film. In this way, we measured the diffraction red-shift for five different serum samples. The human serum samples appear to have an effective physiological buffer concentration of 20 mM[2] based on their response to creatinine.

To use this IPCCA for creatinine sensing for bodily fluids, we can, in the future, either preequilibrate the IPCCA with a solution mimicking the osmolality of bodily fluids or compare the diffraction of the sensor during the determination to that which occurs after all of the creatinine is hydrolyzed and the IPCCA has equilibrated with the exhausted bodily fluids.

We determined the serum creatinine concentration by using a calibration curve for creatinine in Tris−HCl (20 mM) buffered solution containing 150 mM NaCl over the range 0.01−0.7 mM of creatinine concentration. Figure 5 shows that the creatinine concentrations determined in this manner are essentially identical to those determined by using a commercial autoanalyzer (Vitros) which uses the enzyme cascade technique.[5]

Modeling of Creatinine Sensor. A particular creatinine concentration should result in a defined steady-state pH increase within the PCCA hydrogel which leads to a specific extent of phenol deprotonation. The magnitude of phenol deprotonation results in a more favorable free energy of mixing,[33] which results in a diffraction red-shift; the diffraction red-shift will monotonically increase as the creatinine concentration increases.

We can exactly model the response by determining the steady-state value of creatinine within the PCCA. The concentration of creatinine in any PCCA volume element is determined by creatinine diffusion into the volume and by the reaction of creatinine with the CD enzyme. We consider a PCCA at equilibrium with 5 mM TBS buffer solution. When creatinine is quickly mixed into the buffer solution, the rate of increase of creatinine concentration inside the PCCA can be written as

$$\mathrm{d[C}(x)]/\mathrm{d}t = D_\mathrm{C}\,\mathrm{d}^2[\mathrm{C}(x)]/\mathrm{d}x^2 - K_\mathrm{E}[\mathrm{E}][\mathrm{C}(x)] \qquad (1)$$

where [C(x)] is the concentration of creatinine at point x, D_C is the creatinine diffusion constant within the IPCCA (which we calculate to be 9.3×10^{-6} cm^2 s^{-1} in water by using the Stokes−Einstein relation assuming a 2.6 Å hydrodynamic radius), K_E is the second-order rate constant for reaction of the CD enzyme with creatinine, and [E] is the concentration of the enzyme in the IPCCA (93 μM).

We consider one-dimensional diffusion, where x is normal to the surface, because we measure diffraction from the (111) planes which lie parallel to the surface. We monitor diffraction changes due to lattice constant changes along the IPCCA normal. We expose an IPCCA area of ~6 cm^2 and 125 μm thickness to the sample solutions. One side of the IPCCA adheres to the Petri dish, while the other side is exposed to the sample solution.

At steady state (d[C(x)]/dt = 0), the solution of eq 1 is

$$[\mathrm{C}(x)] = ae^{bx} + fe^{-bx} + dx + e \qquad (2)$$

For an infinitely thick PCCA, [C(∞)] is zero; hence $a = d = e = 0$. At $x = 0$, [C(0)] = [C$_0$]; hence f = [C$_0$]. Here, [C$_0$] is the creatinine concentration in a well stirred reservoir in contact with the PCCA. We assume a sufficiently large reservoir volume that [C$_0$] does not change appreciably during the time required to reach steady state. By substituting eq 2 into eq 1:

$$b^2 = K_\mathrm{E}[\mathrm{E}]/D_\mathrm{C} \qquad (3)$$

We determined the value of K_E = 7300 M^{-1} s^{-1} spectrophotometrically by measuring the exponential temporal decay of the creatinine absorbance at 242 nm when a known amount of CD enzyme was added to a known volume of 5 mM TBS buffer solution containing 1 mM creatinine. Thus, the creatinine concentration inside the PCCA at steady state becomes

$$[\mathrm{C}(x)] = [\mathrm{C}_0]\exp -((K_\mathrm{E}[\mathrm{E}]/D_\mathrm{C})^{1/2})x \qquad (4)$$

b is calculated from the values of K_E, [E], and D_C to be 270 cm^{-1}.

The reaction of creatinine with the CD enzyme stoichiometrically produces hydroxide which causes the acid form of the buffer concentration, [B(x)], to decrease and the hydroxylated basic buffer concentration [BOH(x)] to increase, which increases the internal IPCCA pH.

$$B + OH^- \leftrightarrow BOH^-$$

The relative buffer concentrations are constrained by the equilibrium constant, $K_b = [BOH]/[B][OH^-]$. For a buffer with $pK_a = 8.06$, $K_b = 8.71 \times 10^5\ M^{-1}$. For a 5 mM total buffer concentration, at pH 7.4, $[B_0]$ and $[BOH_0]$ are estimated from the Henderson−Hasselbalch expression to be 4.1 and 0.9 mM, respectively.

The presence of creatinine produces hydroxide and hydroxylated buffer within the IPCCA. The excess [BOH(x)] diffuses out of the IPCCA until a steady state is reached where the production and the diffusion loss of [BOH(x)] balance each other.

At steady state, $d[C(x)]/dt = 0$; $d[BOH(x)]/dt = 0$. Hence,

$$D_{BOH}\, d^2[BOH(x)]/dx^2 = K_E[E][C_0] \exp - ((K_E[E]/D_C)^{1/2})x \quad (5)$$

where D_{BOH} is the diffusion coefficient of BOH in the IPCCA. [BOH(x)] can be obtained by integrating eq 5:

$$[BOH(x)] = (D_C[C_0]/D_{BOH}) \exp - ((K_E[E]/D_C)^{1/2})x + mx + n \quad (6)$$

$[C(\infty)] = 0$ requires that production of hydroxide and hydroxylated buffer is zero at $x = \infty$. Hence, $[BOH(\infty)] = [BOH_0]$, where $[BOH_0]$ is the initial BOH concentration both inside and outside the PCCA. This boundary condition gives $m = 0$ and $n = [BOH_0]$.

At $x = 0$, [BOH] just inside the IPCCA near the boundary $x = 0$ is $[BOH_0] - (D_C[C_0]/D_{BOH})$, whereas that just outside the IPCCA is $[BOH_0]$. This results in a small discontinuity in [BOH] at $x = 0$. [BOH(x)] decreases exponentially inside the PCCA with an exponent identical to that of [C(x)]. Thus,

$$[BOH(x)] = (D_C[C_0]/D_{BOH}) \exp - ((K_E[E]/D_C)^{1/2})x + [BOH_0] \quad (7)$$

Because $[B] + [BOH] = 5$ mM, $[B(x)] = 5$ mM $- [BOH(x)]$. Thus,

$$[B(x)] = [B_0] - (D_C[C_0]/D_{BOH}) \exp - ((K_E[E]/D_C)^{1/2})x \quad (8)$$

because

$$[OH(x)] = [BOH(x)]/([B(x)]K_b) \quad (9)$$

We can substitute eqs 7 and 8 into 9, to calculate [OH(x)].

$$[OH(x)] = \frac{(D_C[C_0]/D_{BOH}) \exp - ((K_E[E]/D_C)^{1/2})x + [BOH_0]}{K_b\{[B_0] - (D_C[C_0]/D_{BOH}) \exp - ((K_E[E]/D_C)^{1/2})x\}} \quad (10)$$

The diffracted light observed derives from the first ~30 μm of the IPCCA.[36] Hence, we can roughly calculate the diffracted wavelength observed by assuming it is the wavelength diffracted at the point $x = 15$ μm. We calculated [C(x)], [BOH(x)], [B(x)], and [OH(x)] at $x = 15$ μm using eq 10 to get the "effective pH" inside the probed IPCCA. The corresponding diffraction red-shift was determined from the known pH dependence of the diffraction displayed Figure 2. This calculated diffraction red-shift as a function of creatinine concentration $[C_0]$, for a sample buffer concentration of 5 mM, is shown as the solid line in Figure 3.

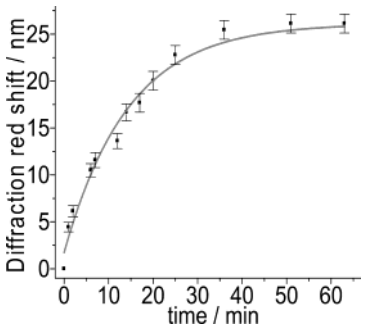

Figure 6. Time dependence of diffraction red-shift of the creatinine sensor IPCCA upon addition of 0.3 mM creatinine (5 mM TBS at pH 7.4). The sensor required approximately 30 min to reach steady state.

The curvature of the calculated fit matches the experimental diffraction red-shifts very well. However, the magnitude calculated is 4-fold smaller than that observed. This may indicate that the actual value for D_{BOH} in the hydrogel is ~4-fold smaller than that in water due to electrostatic interactions of the BOH anion with the negative charges in the hydrogel, which are contributed by the colloidal particles and the bound phenolate anions.

Other possible alternatives are that there is a partitioning of species between the hydrogel and the aqueous reservoir. Except for this 4-fold difference, we also find excellent agreement for the buffer concentration dependence of the diffraction red-shift for a creatinine concentration of 0.5 mM (shown as a solid line in Figure 4). We intend in the future to directly measure the diffusion constants of BOH in the IPCCA and to examine partitioning.

The present model approximates the IPCCA as infinitely thick, which is fine for the 125 mm thickness of the present IPCCA, given the large value of b (270 cm^{-1}). This approximation may fail if we significantly decrease the IPCCA thickness. In the future, we will increase the accuracy of our model by appropriately averaging the diffraction over the entire IPCCA thickness contributing to the diffraction. This is a complex issue which will require careful study due to the fact that diffraction is occurring in the dynamical diffraction regime[37] and that we do not yet know how to include the contribution of CCA defects and the Debye−Waller factor into the diffraction phenomena. However, the fact that the model fits the data well at the average thickness over which we expect diffraction to occur indicates we have captured most of the chemistry and physics. A more sophisticated model will, in the future, allow us to also model the diffraction bandwidths.

The experimentally measured diffraction red-shift for all studied creatinine and buffer concentrations increases exponentially with a time constant of ~860 s as shown in Figure 6. The maximum value remains constant for >10 h. This indicates that we reach steady state in [OH(x)] in ~860 s for this 125 μm thick IPCCA. This should also be close to the time required

(36) Rundquist, P. A.; Kesavamoorthy, R.; Jagannathan, S.; Asher, S. A. *J. Chem. Phys.* **1991**, *95*, 1249−1257.

(37) Zachariasen, W. H. *Theory of X-ray Diffraction in Crystals*; Dover: New York, 1945. Rundquist, P. A.; Photinos, P.; Jagannathan, S.; Asher, S. A. *J. Chem. Phys.* **1989**, *91*, 4932−4941.

to reach steady state for [C(x)], which is the time frame required for creatinine concentration measurements.

Steady state will more quickly be reached in a thinner IPCCA. In fact, we observe a 120 s time constant for 40 μm thick IPCCA.

Conclusions

We developed a new photonic crystal sensor for creatinine which determines creatinine at the concentrations found in human serum. Different creatinine concentrations shift the diffraction wavelength over a span of ~50 nm from 470 to 520 nm (blue to green). The present accuracies and detection limits are similar to those of the conventional clinical methods for determining creatinine. We will continue to refine this sensor to increase its spectral span. The goal is to span the diffraction wavelength shifts from violet to red over the physiologically relevant creatinine concentrations. This would allow the creatinine concentration to be visually determined with the aid of a color chart. This sensor would then be useful as a point-of-care clinical sensor which could be used at the bedside without instrumentation.

This creatinine sensor will be ideal for use by patients with renal disease[2,21] or patients who have undergone kidney transplantation, for home monitoring of bodily fluid creatinine concentrations (ex-situ or in-vivo implanted), to judge the likelihood of the newly transplanted kidney being rejected by their immune systems. The sensor itself is inexpensive and robust and would require no instrumentation. The creatinine concentration should be able to be visually determined from the sensor color by using a creatinine calibration color chart. The long-term health benefits and cost savings to patients with renal dysfunction as well as to healthcare providers could be enormous.

This new sensor motif appears quite general and can be utilized for any analyte which has an enzyme that will react with it to generate H^+ or OH^-. For example, we intend to utilize Jack bean urease to construct a similar PCCA sensor for urea. Because many biological reactions, such as those utilizing pyridine nucleotides, generate or consume a proton, this motif should have broad applicability.

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Supporting Information Available: Experimental details of the determination of 2-nitrophonl concentration and CD enzyme concentration within the creatinine sensor by UV−vis spectroscopy (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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