

Optical Transduction of Chemical Forces

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ABSTRACT

We describe a plasmonic crystal device possessing utility for optically transducing chemical forces. The device couples complex plasmonic fields to chemical changes via a chemoresponsive, surface-bound hydrogel. We find that this architecture significantly enhances the spectroscopic responses seen at visible wavelengths while enabling capacities for sensitive signal transduction, even in cases that involve essentially no change in refractive index, thus allowing analytical detection via colorimetric assays in both imaging and spectroscopic modes.

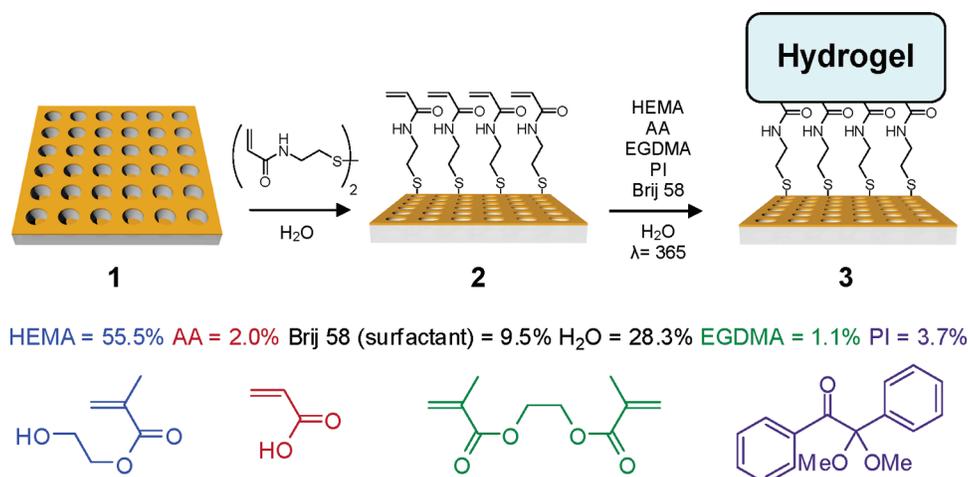
Periodically arrayed nanostructured metallic thin films exhibit near-infrared refractive index (RI) sensitivities in their optical transmission spectra and have been demonstrated to be extremely sensitive to simple surface adsorption of biological macromolecules.¹ This plasmonic response can be extended to include a variety of analytically relevant parameters using a surface-attached hydrogel containing specific receptors that induce a polymer density change in response to an external stimulus.² By transducing events not associated with dramatic differences in RI into large plasmonic responses, these hydrogel-modified plasmonic crystals (PC) can optically couple and detect events that are traditionally measured using less convenient means. This work demonstrates a hydrogel-modified plasmonic crystal containing receptor groups that are sensitive to analytes that cause changes in the charged state of the hydrogel. This results in a relative shift in the chemical equilibrium intrinsic to the cross-linked network (swelling or deswelling) that is manifested to first order as a RI change in the hydrogel thin film. Coupling the swelling dynamics of the hydrogel to the resonant modes of the plasmonic crystal makes it possible to optically measure the chemical forces (osmotic pressure) associated with changes in the pH of the surrounding solution by observing shifts in the plasmonic resonance energies and intensities. The surface modification of the PC device also leads to marked impacts on its spectroscopic response, eliciting sensitivities of these devices not only in the near-infrared but also the visible. These optical impacts are shown to readily allow for efficient large-area imaging-based detection of osmotic pressure gradients throughout a hydrogel thin film using a simple apparatus.

Recent advances in nanofabrication capabilities have given rise to numerous array-based plasmonic RI sensing schemes that use simple collinear transmission (or reflection) optical geometries.^{3–5} These nanostructured devices come in a variety of form factors, e.g., metal nanoparticle,⁶ nanohole,⁷ or nanowell arrays,⁵ all of which exhibit significant electric field enhancements on or near the nanostructured features.⁸ The plasmon resonances seen in these various devices are sensitive to RI changes at the dielectric interface and can be tuned in sensitive ways by the design rule of their periodic structures as well as through manipulation of minute structural details of their nanoscale features.⁹

The plasmonic crystals used in this work consisted of embossed, Au-coated nanowell arrays fabricated using a procedure that has been described previously.⁵ The design of the device (Scheme 1) is based on a square array of ca. 350 nm deep cylindrical impressions in a thin film of Norland Optical Adhesive 73 (NOA 73) with a pitch and diameter ca. 780 and 480 nm, respectively.⁵ To provide a stable sensor response, the embossed polymer film was immersed in a solution of neat ethanethiol for one week to terminate any residual reactive functionalities in the cured NOA 73 network. The slides were then rinsed, and residual solvent and thiol were removed under reduced pressure (>24 h). A metallic thin film of gold (50 nm) was then evaporated onto the nanostructured surface (Scheme 1, **1**). Transmission spectra of **1** exhibit multiple peaks in both the visible and near-infrared regions. The latter regions are highly sensitive to changes in refractive index at the surface of the device, the former less so.¹ Figure 1A shows transmission spectra of **1** taken in buffer solutions at pH = 7.86 and 1.44. Little change is noted in these spectra, as expected based on the

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Scheme 1. Fabrication of a Surface-Bound pH Responsive Hydrogel on a Plasmonic Crystal



negligible differences in the refractive indices of these two solutions ($<1 \times 10^{-4}$ refractive index units).¹⁰

A hydrogel thin film was then chemically bound to the surface of the plasmonic crystal using an acrylamide-terminated self-assembled monolayer (SAM) as an anchor (Scheme 1, 2). This monolayer prevents the hydrogel film from pulling away from the gold surface as it swells. The polymer was formed using a thin film of a solution composed of 2.0 wt % acrylic acid (AA), 1.1 wt % ethylene glycol dimethacrylate (EGDMA) cross-linker, 3.7 wt % photoinitiator (PI), 55.5 wt % hydroxyethyl methacrylate (HEMA), and aqueous Brij 58 surfactant.¹¹ This AA composition is lower than that used in earlier hydrogel work to attenuate the gel volume change and facilitate film adhesion.^{11,12} The monomer solution (ca. 20 μ L) was pressed between the PC and a glass slide and then polymerized using a UV light source to produce a hydrogel thin film ca. 500 nm thick

(Scheme 1, 3).¹⁰ After removing the microscope slide, the device was placed into a flow cell and aqueous analyte solutions were introduced.¹⁰

Equilibration of 3 in a basic aqueous buffer solution causes full expansion of the hydrogel. To remove any effects of ionic strength from the hydrogel swelling, all solutions were adjusted to an ionic strength of 0.2 M with NaCl. Phosphate-citrate buffered solutions were used as the analyte solutions to expose the gel to a variety of pH values spanning the pK_a of acrylic acid. Transmission spectra at pH 7.86 show numerous broad resonances throughout the visible-NIR spectrum (Figure 1B). This solution was chosen as the most basic solution to study as it is below the pK_a of any free thiols (pK_a ca. 9) present in the bulk of the NOA 73 polymer. Changing the solution to pH 1.44 collapses the hydrogel (control experiments show volumetric contractions of ca. 27% are obtained)¹⁰ and results in significant changes being seen in both the energies and intensities of the transmission spectra (Figure 1B). These responses are over the entire visible-NIR region and are significantly stronger than responses from nonmodified PCs of this type, as seen in the essentially indistinguishable spectra for 1 (Figure 1A).¹⁰ The marked changes for 3 (Figure 1B) are associated with plasmonic interactions with the pH responsive dynamics of the hydrogel film.

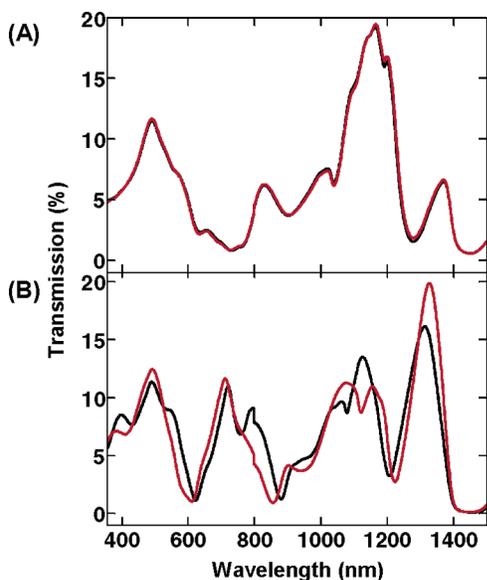


Figure 1. Transmission spectra of a plasmonic crystal in a basic pH 7.86 buffer solution (black) and an acidic pH 1.44 buffer solution (red). (A) Crystal 1, no hydrogel; (B) Crystal 3, with hydrogel.

A series of difference spectra (as referenced to an initial transmission spectrum at time = 0) demonstrate both the reversibility and stability of hydrogel-driven plasmonic responses (Figure 2A). No changes are observed in the transmission spectra while a constant pH 7.86 analyte solution is continuously flowed for 1 h. Switching the solution pH to 1.44 results in a broad spectroscopic response that is both substantial in its magnitude and fully reversible upon cycling between these two limiting pH values.¹³ The data in Figure 2 illustrates this point using representative response curves taken from a sequence of nine complete pH cycles. In a separate control experiment, a PC bearing an immobilized hydrogel that contains no AA residues shows a greatly attenuated response at all wavelengths.¹⁰

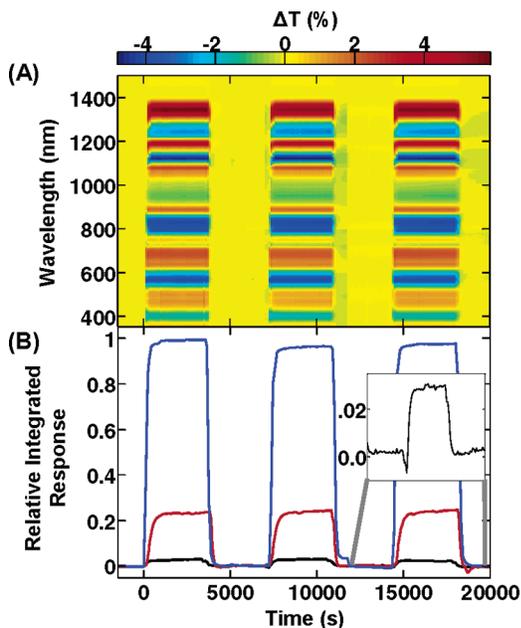


Figure 2. (A) Spectral sensitivity map consisting of difference spectra from crystal **3** referenced to $t = 0$ s, after which the analyte solution is cycled between pH 7.86 and 1.44. (B) The integrated plasmonic response corresponding to reversible changes in analyte solution from pH 7.86 to 1.44 (blue), 6.42 to 5.13 (red), and 5.76 to 5.66 (black). The smallest pH change (0.10) is well differentiated from the stable background signal (inset).

Summing the absolute magnitudes of the difference spectra over all frequencies yields an integrated plasmonic response¹ that directly correlates with pH-dependent changes in the properties of the hydrogel film. The sequence of large pH changes ($\Delta\text{pH} = 6.42$) in Figure 2A correlates to a relatively large integrated response (Figure 2B). A decrease in the pH change ($\Delta\text{pH} = 1.29$) centered around the experimentally determined $\text{p}K_a$ of the hydrogel (vide infra) leads to retention of ca. 25% of the integrated response. Further reduction of the pH change shows a drastic attenuation in the integrated response (ca. 3% of the original response). From these data, it is apparent that changes of 0.1 pH unit are readily discernible from the background (inset Figure 2B), with an ultimate noise limited lower limit of detection being ca. 1×10^{-4} pH units. These results illustrate the sensitivity of these systems even though they are driven by an extremely dilute concentration of acid groups in the hydrogel. We believe this constitutes a more general form of chemical amplification that can eventually be extended to other types of receptor groups incorporated within the hydrogel.¹⁴

In the current work, we examined the utility of this system for making direct measurements of pH, chemical forces, and chemical imaging. The $\text{p}K_a$ of the hydrogel was experimentally determined using the integrated plasmonic response of **3** to create a classic titration curve with a series of increasingly basic solutions. Each solution was allowed to equilibrate for 30 min, resulting in a quantized integrated response with each step corresponding to a new pH value (Figure 3A). The integrated response is limited by the kinetics associated with the hydrogel swelling/deswelling and, as such, requires equilibrium to be reached in order to make

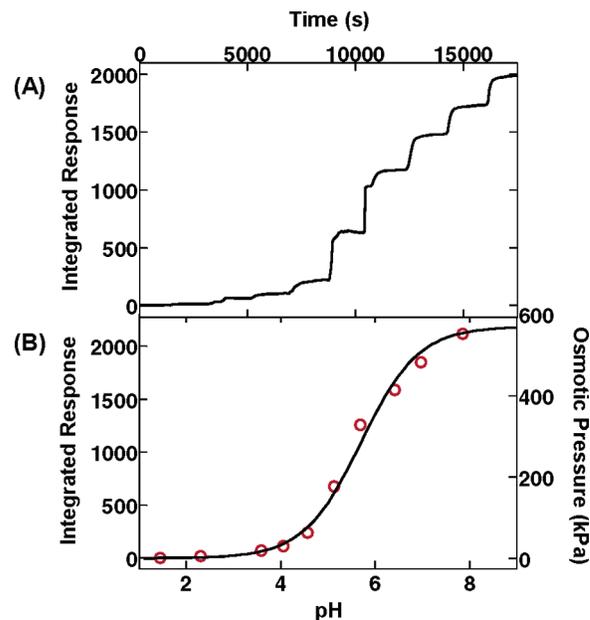


Figure 3. (A) Integrated response of crystal **3** upon exposure to solutions at pH 1.44, 2.31, 3.59, 4.08, 4.57, 5.13, 5.66, 6.42, 6.95, and 7.86 in that order for 30 min each. (B) Average integrated response after equilibrating for 30 min at each particular pH (left) along with the calculated osmotic pressure experienced by the gel network at each pH (right). The best fit line to these data (black) yield a hydrogel $\text{p}K_a$ of 5.70.

accurate static osmotic pressure measurements. The equilibrium value for each step plotted against the pH of the solution yields a titration curve that can be fit to the polymer-specific form of the Henderson–Hasselbalch equation (eq 1).¹⁵ The fit parameters indicate a $\text{p}K_a$ of 5.70 and n (the activity correction which accounts for the $\text{p}K_a$ distribution of the AA

$$\text{pH} = \text{p}K_a - n \left(\frac{\alpha}{1 - \alpha} \right) \quad (1)$$

groups in the polymer)¹⁵ of 1.4, which are consistent with previous studies of AA-containing polymer networks.¹⁶

The physics associated with the observed pH responses in hydrogels of this type is known to involve swelling/deswelling of the loosely cross-linked polymer network.¹⁷ To first order, these volume changes are the direct result of a shift in the equilibrium between the osmotic forces acting to swell the hydrated gel and the covalent bonds in the polymer, holding the network intact. The hydrogel films are thicker than the penetration depth of the electric fields associated with the plasmonic modes supported by the PC devices.¹ The observed responses are therefore an indirect measure of hydrogel volume changes, which can be correlated to an effective osmotic pressure (P_{osmotic}) using eq 2, where c_k^o and c_k are the concentration of the k th ion in the solution outside and inside of the hydrogel, respectively.^{18,19}

$$P_{\text{osmotic}} = RT \sum_{k=1}^N (c_k - c_k^o) \quad (2)$$

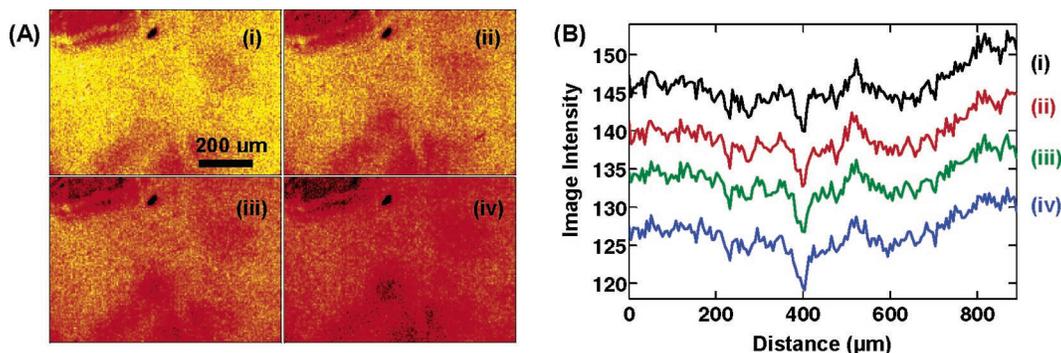


Figure 4. (A) Imaging response of an equilibrated crystal **3** (pH 1.44) at various times after exposure to a basic solution (pH 7.86): (i) $t = 0$ s, (ii) $t = 100$ s, (iii) $t = 175$ s, (iv) $t = 275$ s. (B) Average intensity of each vertical column of image pixels in A.

The osmotic pressure changes can be calculated for the transition between the swollen and the collapsed states by estimating the fixed charge density in the gel.²⁰ Figure 3B shows the titration curve derived from the integrated response data along with the correlated osmotic pressure change associated with the gel network. We note that, using the plasmonic crystal in this manner, it is possible to measure mechanical forces exerted on the hydrogel by even small concentration gradients present in the hydrating solution. In this case, proton gradients are the activating mechanism; however, this detection scheme could be tailored to any number of analytes using specific recognition elements incorporated into the hydrogel network that elicit a gel volume change.¹⁴

An important aspect of these surface-modified plasmonic crystals is that the visible region of the transmission spectra show a markedly enhanced sensitivity to the forces associated with the swollen and deswollen states of the hydrogel. The possibility for detecting such changes in an imaging mode was demonstrated using a visible CCD camera at $5\times$ magnification to image a sample of **3** with a $890\ \mu\text{m} \times 440\ \mu\text{m}$ area. Figure 4A shows large changes in the overall color intensity transmitted through **3** as the pH of the solution is changed from pH 1.44 to 7.86. Averaging the intensity of each vertical column of the image pixel array results in an average horizontal image intensity that is fairly uniform over the width of the imaging area. This averaged image intensity clearly decreases as a function of increasing pH (Figure 4B). One defect feature appears in these images as a dark spot that did not change intensity as a function of pH. This internal standard was used as a calibration for the light source and demonstrates that image intensity variations seen are due to hydrogel dynamics and not light source fluctuations.¹⁰

The sensitivity enhancements seen at visible wavelengths appear to reflect unique optical features of the modified plasmonic crystals. Rough estimates of the index changes that attend gel collapse (and expansion) are too small to fully capture the effects seen experimentally. Other factors must be invoked. We believe that the most likely origin lies in the gel acting to generate an optical cavity that serves to elicit a strongly wavelength-dependent modulation of the plasmonic response. Quantitative theory-based models are currently being developed to describe these behaviors and will be subsequently reported.

In summary, a hydrogel-modified plasmonic crystal has been shown to effectively transduce chemical forces to optical responses. This novel sensing scheme responds strongly to small changes in hydrogel volume density and produces broad multiwavelength responses spanning the visible and near-infrared regions, even in cases involving negligible changes in refractive index. We believe this work offers opportunities for numerous receptor-based chemical recognition schemes, a prospect that is the focus of future work.

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Supporting Information Available: Detailed experimental procedures and additional experiments. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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