ChemComm

RSC Publishing

COMMUNICATION

View Article Online
View Journal | View Issue

# Two-dimensional array Debye ring diffraction protein recognition sensing†

Cite this: *Chem. Commun.*, 2013, **49**, 6337

Received 7th May 2013,
Accepted 24th May 2013

DOI: 10.1039/c3cc43396j

www.rsc.org/chemcomm

Jian-Tao Zhang, Xing Chao, Xinyu Liu and Sanford A. Asher*



**We attach 2-D colloidal arrays onto the surface of hydrogels containing biotin. The hydrogel volume shrinks with increasing concentrations of avidin due to the formation of avidin–biotin crosslinks. This causes the Debye diffraction ring diameter to increase, and the 2-D diffraction wavelength to blue-shift.**

Diagnosis of disease often requires the identification of specific marker proteins. Methods such as enzyme-linked immuno sorbent assay (ELISA) and LC-MS have been developed to rapidly identify specific proteins.[1–3] Unfortunately the complexity of these protein detection techniques limits their utility. In the work here we are developing photonic crystal sensing methodologies that are simple and could be used for the visual detection of proteins from complex aqueous samples.

Two-dimensional (2-D) crystalline colloidal arrays (CCA) have numerous applications, such as photonic crystal materials, sensors, *etc.*[4–7] Recently we developed polymerized CCA (PCCA) sensing materials by attaching 2-D monolayer arrays of particles onto responsive hydrogels.[5,6] These 2-D PCCA show strong forward diffraction that we can use to visually detect analytes. The forward 2-D diffraction can be monitored by using a mirror to back reflect the forward diffracted light.[6,7]

This diffracted light can be measured by using a reflection probe in a Littrow configuration. The diffracted wavelength follows: $m\lambda = 3^{1/2}d \sin\theta$, where $m$ is the diffraction order, $\lambda$ is the wavelength in air, $d$ is the nearest neighboring particle spacing, and $\theta$ is the angle between the incident light and the 2-D array normal.[5–7] We controlled $\theta$ and $d$ to achieve diffraction in the visible region.

Here, we report the development of a photonic crystal 2-D protein sensing hydrogel containing biotin that binds strongly to the protein avidin.[8,9] Avidin binding forms new hydrogel crosslinks that shrink the hydrogel. The decreased 2-D particle spacing can be read out from the diffracted light color changes. Swelling of the hydrogel increases $d$, which shifts the diffraction towards the IR. This makes it difficult to observe the diffraction. We demonstrate that these 2-D array spacings can be easily measured by monitoring the forward 2-D array diffraction that forms a Debye ring.[10,11]

We fabricated the 2-D array hydrogel sensor by polymerizing a hydrogel on top of a close-packed hexagonal 2-D particle array and then attaching biotin to the hydrogel (Fig. 1a). We fabricated the 2-D 650 nm PS particle array on a water surface by using our needle tip flow method[12] and then transferred the 2-D array onto a glass slide (2.4 × 6.0 cm).

A mixture of 8 mg *N*-hydroxysuccinimide ester acrylic acid (NHA), 400 μL acrylamide (AAm) aqueous solution (10 wt%) with 0.1% *N*,*N*′-methylenebisacrylamide (MBAAm), and 8 μL of an Irgacure 2959/DMSO solution (33% w : v) was layered onto a 2-D array on the glass slide. The reaction solution contacting the 2-D array was covered by another glass slide (2.4 × 6.0 cm) and the sample was photo polymerized for 10 min at room temperature by using a UVGL-55 UV Lamp (UVP, CA). The 2-D particles were embedded within the PAAm-NHA hydrogel.

We peeled the 2-D array hydrogel from the glass slide and washed it with a 0.1 M NaCl aqueous solution for 30 min. Then we incubated the PAAm-NHA 2-D array film overnight in 6.5 mL of a 0.1 M NaCl solution containing 30 mg of an EZ-Link Amine-$PEG_3$-Biotin solution (4.6 mg $mL^{-1}$) to attach the biotin. This chemistry was previously used to couple proteins onto hydrogels.[13] The unreacted NHA was capped with ethanolamine and the system was washed with a 0.1 M NaCl solution (Fig. 1b).

The SEM (Fig. 2a) of the 650 nm PS array on a glass slide shows that the 2-D particles form a close-packed hexagonal array. Fig. 2b shows that during hydrogel polymerization the space between neighboring particles fills with hydrogel polymer. Fig. 2c shows the SEM image of an abraded area of the 2-D array hydrogel, demonstrating that the 2-D array is a monolayer situated on top of the hydrogel.

We were able to dissolve the PS 2-D array particles with ethyl acetate leaving a 2-D array of holes (Fig. 2d), indicating that the monomer solution infiltrates around the 2-D particles. After polymerization, the particles are embedded in the hydrogel, not only on the surface.

2-D array photonic crystal sensing hydrogels have significant advantages over previous 3-D sensing hydrogels. A major advantage

*Department of Chemistry, University of Pittsburgh, Pittsburgh, PA, 15260, USA. E-mail: asher@pitt.edu*

† Electronic supplementary information (ESI) available: Experimental section and photograph showing the 2-D PS array biotin hydrogel diffracted colors in pure water and in 0.1 M NaCl. See DOI: 10.1039/c3cc43396j

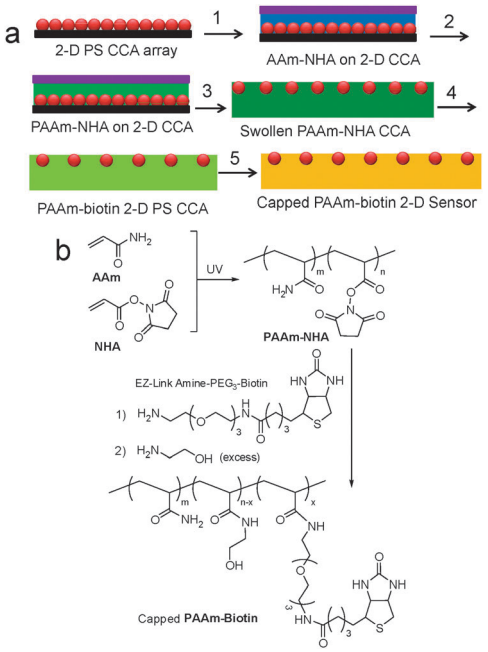


**Fig. 1** (a) Preparation of 2-D array hydrogel avidin sensor: 1. layer AAm and NHA solution on 2-D array on glass; 2. photopolymerize AAm and NHA solution into hydrogel with UV light; 3. peel 2-D array PAAm-NHA hydrogel film from glass and wash with 0.1 M NaCl; 4. attach biotin; 5. cap unreacted NHA with excess HO–$CH_2CH_2$–$NH_2$. (b) Hydrogel fabrication chemistry, including copolymerization of AAm and NHA, attachment of biotin and capping of unreacted NHA with ethanolamine.

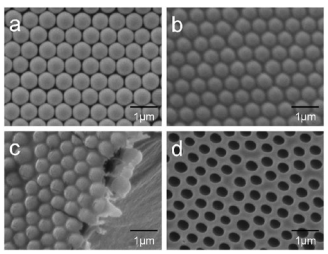


**Fig. 2** SEM of (a) PS 2-D array on glass; (b) PS 2-D array monolayer on top of PAAm-NHA hydrogel, where interstices between particles are filled with polymer; (c) abraded hydrogel showing PS 2-D array on PAAm-NHA hydrogel; and (d) 2-D hole array on hydrogel after dissolving particles with ethyl acetate, leaving a 2-D hole array on hydrogel.

is that formation of the sensing hydrogel is decoupled from the array assembly and ordering. 3-D particle array ordering requires exclusion of ions. Here the 2-D CCAs are attached to a solid substrate. Any functional monomers can be polymerized into the hydrogel. For example, we were able to prepare 2-D array poly(methacrylic acid) and poly(2-hydroxyethyl methacrylate) hydrogels. Further, 2-D arrays enable very thin photonic crystal sensors for sensing surface species.

Binding of avidin to the hydrogel biotins forms crosslinks that shrink the hydrogel. The 2-D particle spacings decrease, causing the 2-D array diffraction to blue shift. Fig. 3a shows the diffraction spectra of the 2-D PCCA biotin hydrogel sensors in different concentrations of avidin in 0.1 M NaCl. The diffraction wavelength blue shifts with increasing avidin concentrations. For example, the diffraction shifts from 617 nm to 550 nm for avidin concentration increases from 0 to 1 mg $mL^{-1}$. Fig. 3b shows the dependence of the diffraction wavelength maximum on the avidin concentration. The Fig. 3b inset shows that the diffraction of the 2-D biotin sensors

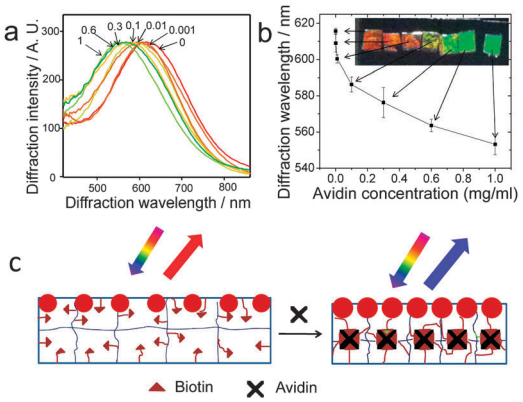


**Fig. 3** (a) Dependence of normalized diffraction spectra of 2-D biotin hydrogel sensors upon avidin concentration in 0.1 M NaCl aqueous solution. Diffraction is measured in a Littrow configuration with an angle between the probe and the 2-D array normal of ~19°; (b) dependence of diffraction wavelength on avidin concentration. Inset shows photographs taken close to the Littrow configuration at an angle of 19° between the light source/camera and the 2-D array normal; (c) schematic showing avidin molecule binding to multiple hydrogel biotins. This increases the crosslinking, blue shifting the diffraction.

shifts from red to yellow, and then to green with increasing avidin concentrations. We are able to visually detect the presence of 0.1 mg $mL^{-1}$ avidin where the diffraction color changes from red to yellow green (Fig. 3b inset).

The blue shift is caused by the strong avidin–biotin binding crosslinks formed in the hydrogel sensors. Avidin has four extraordinarily high affinity binding sites for biotin ($K_a$ = $10^{15}$ $M^{-1}$).[14] These crosslinks shrink the hydrogel, decreasing the 2-D array spacing, blue shifting the 2-D diffraction (Fig. 3c).

We used bovine serum albumin (BSA) as a protein control to demonstrate that avidin selectively binds to biotin attached to the hydrogel. We put a biotin 2-D array hydrogel in 0.1 mg $mL^{-1}$ BSA in 0.1 M NaCl. We did not find any diffraction change, indicating no binding between BSA and biotin. We also investigated the avidin response of a biotin-free 2-D array hydrogel that was blocked by ethanolamine. No diffraction shifts were observed with increasing avidin concentrations up to 1 mg $mL^{-1}$. This shows that the biotin hydrogel shrinkage in the presence of avidin is caused by specific avidin–biotin recognition.

The 2-D array biotin hydrogel swells when transferred from 0.1 M NaCl to water, causing an increase in the particle spacing due to the increased Donnan potential. We cannot visually observe the diffraction of the 2-D array hydrogel in water because the diffraction shifts to the near IR region (as shown in ESI,† Fig. S1). The diffraction also cannot be measured by a visible wavelength spectrometer. However, we can detect the hydrogel 2-D array spacing changes by monitoring the forward 2-D array diffraction.

2-D CCA show strong forward light diffraction. For normal incidence, a perfectly ordered 2-D array shows six diffraction spots for monochromatic light. At normal incidence, the diffraction pattern on a screen indicates the 2-D particle spacing and ordering.[10,11] For example, Velev *et al.* used diffraction to investigate the colloidal particle electric field induced 2-D self-assembly.[11] Liddell *et al.* used the pattern to study the assembly of nonspherical dimers of particles.[10c] An elliptical ring pattern was observed from a particle dimer polycrystalline structure. The diffraction changed to sharp spots when the laser beam diffracted from a single domain.[10c]

 

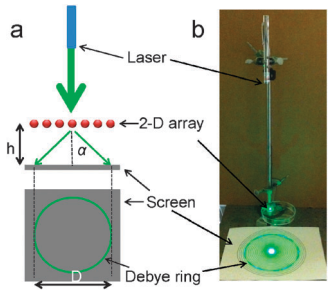


**Fig. 4** (a) Illustration of Debye diffraction ring measurement. *h* is distance between the 2-D array plane and the screen. *D* is the diameter of the diffraction ring on screen. The diffraction angle, $\alpha$, is calculated from $\tan \alpha = D/2h$; (b) photograph showing the Debye diffraction ring from a 1.26 μm diameter PS 2-D array for a 532 nm excitation wavelength.

Fig. 4a schematically shows our Debye ring diffraction measurement geometry. We excite the array along its normal with a 532 nm laser. The 2-D array diffracts light at specific angles that depend on the particle spacing and the laser wavelength. If the beam illuminates a single domain hexagonal 2-D array, diffraction shows six spots on the screen. For randomly oriented 2-D array domains smaller than the size of laser beam, the diffraction pattern of the 2-D array will give a ring.[11] This is because the 2-D array domains in the illuminated area have identical spacings but random orientations. These domains show many spots with the same diffraction diameter, forming a ring.

Fig. 4b shows that a 1.26 μm nearest neighboring spacing PS 2-D array illuminated by a 532 nm green laser pointer shows a Debye diffraction ring of diameter *D*, of 15.0 cm for a distance between the 2-D array and screen, *h* of 13.4 cm. The brighter regions on the ring result from domains of larger size.

As shown in Fig. 4a, the first-order diffraction angle, $\alpha$, depends upon the particle spacing: $\sin \alpha = 2\lambda_{laser}/(3^{1/2}d)$, where $\alpha$ is the interior angle of the Debye diffraction ring, $\lambda_{laser}$ is the laser wavelength, and *d* is the particle spacing.[10,11] The diffraction angle $\alpha$, can be determined from the Debye diffraction ring diameter: $\alpha = \tan^{-1}(D/2h)$. We easily monitor the 2-D particle spacing by measuring the Debye ring diameter.

Debye ring diffraction measurement has the advantage that it does not require a spectrometer and the instrumentation is inexpensive. The Debye diffraction ring method does not require careful control of the incident and diffracted light angles. This is especially important for delicate samples that are hard to handle.

This method allows us to easily monitor the 2-D array spacings of hydrogels with large particle spacings that diffract in the IR. Fig. 5 shows the dependence of the 2-D array spacing of a biotin hydrogel on avidin concentration in pure water determined from the Debye ring diameter. The particle spacing decreases from 2290 nm to 1710 nm as the avidin concentration increases from 0 to 1 mg $mL^{-1}$. The 2-D array hydrogel sensor sensitivity of 580 nm/(mg $mL^{-1}$) avidin in water is much larger than in 0.1 M NaCl (110 nm/(mg $mL^{-1}$)), where the particle spacing shifts from 1090 nm to 980 nm upon an avidin concentration increase from 0 to 1 mg $mL^{-1}$.

We have extended this protein recognition sensing to analyte proteins with smaller association constants. For example, we

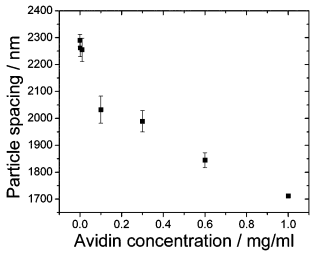


**Fig. 5** Dependence of 2-D array spacing of biotin hydrogels on avidin concentration in pure water.

recently demonstrated sensing of proteins with much lower binding affinities ($0.82 \times 10^4$).[15] We developed photonic crystal sensors for Concanavalin A by attaching mannose to 2-D array hydrogels.[16]

In summary, we fabricated a 2-D array photonic crystal avidin sensing material by photopolymerizing a hydrogel on top of a 2-D PS particle array. These hydrogels were functionalized by attaching biotin that selectively binds avidin. Binding avidin to biotin ligands increased the hydrogel crosslinking and decreased the hydrogel volume and the 2-D particle spacing. The protein binding can be detected by 2-D diffracted light color changes and by 2-D forward Debye ring diffraction. Both readout methods can be easily applied to 2-D array molecular recognition systems for protein–ligand binding detection.

We acknowledge funding from HDTRA (Grant No. 1-10-1-0044). X.L acknowledges financial support from the University of Pittsburgh.

## Notes and references

1 Z. Yang, M. Hayes, X. Fang, M. P. Daley, S. Ettenberg and F. L. S. Tse, *Anal. Chem.*, 2007, **79**, 9294.
2 W. Shi, J. He, H. Jiang, X. Hou, J. Yang and J. Shen, *J. Agric. Food Chem.*, 2006, **54**, 6143.
3 A. Végvári and G. Marko-Varga, *Chem. Rev.*, 2010, **110**, 3278.
4 Y. Li, G. Duan, G. Liu and W. Cai, *Chem. Soc. Rev.*, 2013, **42**, 3614.
5 (*a*) J. T. Zhang, L. Wang, J. Luo, A. Tikhonov, N. Kornienko and S. A. Asher, *J. Am. Chem. Soc.*, 2011, **133**, 9152; (*b*) J. T. Zhang, N. Smith and S. A. Asher, *Anal. Chem.*, 2012, **84**, 6416.
6 J. T. Zhang, L. Wang, X. Chao, S. S. Velankar and S. A. Asher, *J. Mater. Chem. C*, 2012, DOI: 10.1039/c2tc00794k.
7 A. Tikhonov, N. Kornienko, J. T. Zhang, L. Wang and S. A. Asher, *J. Nanophotonics*, 2012, **6**, 063509.
8 M. Wilchek, E. A. Bayer and O. Livnah, *Immunol. Lett.*, 2006, **103**, 27.
9 O. Livnah, E. A. Bayer, M. Wilchek and J. L. Sussman, *Proc. Natl. Acad. Sci. U. S. A.*, 1993, **90**, 5076.
10 (*a*) F. Pan, J. Zhang, C. Cai and T. Wang, *Langmuir*, 2006, **22**, 7101; (*b*) I. D. Hosein and C. M. Liddell, *Langmuir*, 2007, **23**, 8810; (*c*) I. D. Hosein and C. M. Liddell, *Langmuir*, 2007, **23**, 10479.
11 (*a*) B. G. Prevoi and O. D. Velev, *Langmuir*, 2004, **20**, 2099; (*b*) S. O. Lumsdon, E. W. Kaler and O. D. Velev, *Langmuir*, 2004, **20**, 2108.
12 J. T. Zhang, L. Wang, D. N. Lamont, S. S. Velankar and S. A. Asher, *Angew. Chem., Int. Ed.*, 2012, **51**, 6117.
13 (*a*) H. K. A. Tsai, E. A. Moschou, S. Daunert, M. Madou and L. Kulinsky, *Adv. Mater.*, 2009, **21**, 656; (*b*) M. A. Mazumder, S. D. Fitzpatrick, B. Muirhead and H. Sheardown, *J. Biomed. Mater. Res., Part A*, 2012, **100**, 1877; (*c*) R. L. Schnaar and Y. C. Lee, *Biochemistry*, 1975, **14**, 1535.
14 N. M. Green, *Adv. Protein Chem.*, 1975, **29**, 85.
15 (*a*) D. K. Mandal, N. Kishore and C. F. Brewer, *Biochemistry*, 1994, **33**, 1149; (*b*) D. N. Moothoo and J. H. Naismith, *Glycobiology*, 1998, **8**, 173.
16 J. T. Zhang, D. Kwak, X. Liu, and S. A. Asher, in preparation.