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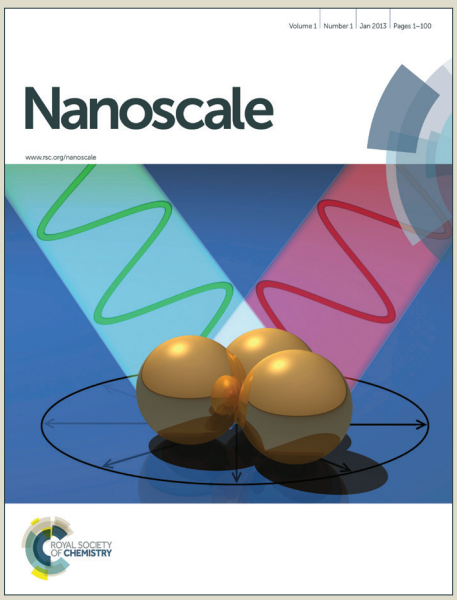

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Colorimetric logic response based on aptamer functionalized colloidal crystal hydrogels†

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A novel colorimetric logic system based on the aptamer-cross-linked colloidal crystal hydrogels (CCH) was developed. With input stimuli of Hg^{2+} and Ag^{+}, the CCH displayed shrinking response and colour change corresponding to the logical "OR" and "AND" gate. The visualization of the logic output signals is realized.

DNA logic gates, as a promising substitute of the traditional silicon-based electronic technologies, have attracted significant research interest. They can generate "outputs" based on the unique biological and physical features of DNA molecules. This imparts the technology with several advantages, involving strand displacement, flexibility in design and the ability of chemical modification.[1] Recently, various DNA logic gates have been developed, which prove it's an ideal candidate that satisfy logic operations.[2-10] Most of these logic gates employed fluorescent,[11-13] electrochemical,[14,15] or electrochemiluminescent[16] signals as their outputs. This makes them suffer from cumbersome handling procedures and instrument-dependent readout. To solve these problems, stimuli-responsive DNA hydrogels, which could transform the output signals to visible hydrogel volume changes, have been suggested as alternative logic gates.[17-20] This strategy simplified the analysis procedure and thus held great promise for low-cost and rapid readout for on-site detection.[21] However, the output signals based on the physicochemical response of hydrogels, such as changes in refractive index and volume, are difficult to measure exactly. Therefore, hydrogel based DNA logic gate with the simple and accurate outputs are still anticipated.

In this research, we propose a novel colorimetric DNA hydrogel logic gate system by incorporating colloidal crystal into a responsive aptamer cross-linked hydrogel. Colloidal crystals, which can be quickly assembled from monodisperse nanoparticles, have long been used to construct optical and sensing devices. According to the Bragg's law ($\lambda = 2nd\sin\theta$; λ is diffraction wavelength, d is lattice plane spacing, n is the average refractive index and θ is the Bragg angle), the periodic variation in the refractive index of them gives rise to interesting optical properties, such as photonic band gaps (PBGs) and structural colours.[22-26] Particularly, when they are combined with stimuli-responsive hydrogel, the hydrogel swelling or shrinking upon application of a stimulus would lead to shifts of the PBGs and changes of the structural colours of the colloidal crystals.[27-33] The displayed colours of our aptamer cross-linked colloidal crystal hydrogels (CCH) could be adjusted by using different target stimuli to trigger the CCH with partial or complete shrinkage, which corresponding to the basis output for the logic "OR" or "AND". Thus, the logical system of CCH presented here performs specific binary visual response. This feature makes it ideal candidate for the construction of intelligent point-of-care devices and molecular logic systems for on-site applications.

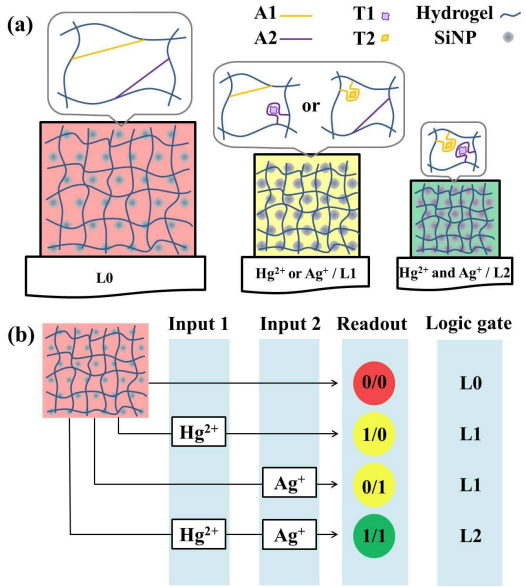


Fig. 1 (a) Schematic illustration of the aptamer functionalized CCH logic swelling triggered by two kinds of input targets. (b) Multiple logic swellings based on the aptamer functionalized CCH, with Hg^{2+} and Ag^{+} as two inputs.

As a proof of concept, the Hg^{2+} and Ag^{+} responsive aptamers (A1 and A2) cross-linked hydrogels were employed as the scaffold polymers of the colorimetric logic CCH.[34, 35] The working principle of our method is illustrated in Fig. 1. The two aptamers A1 and A2 are chemically coupled onto the hydrogel network. The reversible binding between the specific target ion and the aptamer in the hydrogel network could cause the aptamer conformation change and thus triggered the shrinkage of the hydrogel with different content.

Thus, the target Hg^{2+} and Ag^{+} could be used as the input signals to trigger the logic CCH with different shrinkage responsive. In this process, the logic CCH could report a visual output signal of logically "OR" or "AND" depending on the structure colours of the CCH. As schemed in Fig. 1b, in the absence of the targets (0,0), the cross-linked aptamers do not interact with each other, thus the CCH displays its original red colour and reports the initial signal level L0. When one of the targets (T1 or T2) is present, the binding reaction of the aptamer to the single target causes the hydrogel of the CCH with moderate shrinkage, which is reported as yellow colour appearance of output signal level L1. With the present of both T1 and T2 (1,1), the shrinkage of the CCH reaches a higher proportion and the PBG can be shifted to green (output signal level L2) by a complete conformation change of the A1 and A2. Thus, upon at least one of the targets (T1 or T2) inputting (1,0; 0,1; 1,1), the signal ''1'' is obtained when the signal is greater than the output signal level L1 and the logic "OR" gate is realized. Both targets are necessary to get ''1'' value of the output signal greater than the signal level L2 at which logic "AND" gate is accomplished. Therefore, we defined different output signals as the logical value true or false and realized two types of logic gates in one system. Either or both inputs can induce different colours of the CCH and the CCH could be designed to give an "OR" or "AND" logic gate at two distinctly different signal colour levels.

The strategy of our colorimetric DNA logic gate relied essentially on the stimuli responsive of CCH. To fabricate the CCH with a high-quality, monodisperse silica nanoparticles were well dispersed in the pre-gel solution with a relatively high concentration, following a strict ion exchange process. After these treatments, the nanoparticles self-assemble into non-close-packed colloidal crystal array structures in the solution and display with brilliant structural colours, which could be controlled by using different concentrations or sizes of nanoparticles. After polymerizing the pre-gel solution by UV light, the ordered nanostructure of the colloidal crystal array was well locked by a hydrogel network. This was confirmed by scanning electron microscope (SEM) images in Fig. 2, which indicated a long-range order of the alignment of silica nanoparticles both on the surface and inner of the CCH. Due to the ordered structure of the silica nanoparticles, the prepared CCH also showed vivid structural colour for the visual logic observation. Here, we prepared the CCH with a red structural colour of the reflection wavelength at 625 nm for the following research, because the CCH would undergo a blue shift during the logic shrinkage.

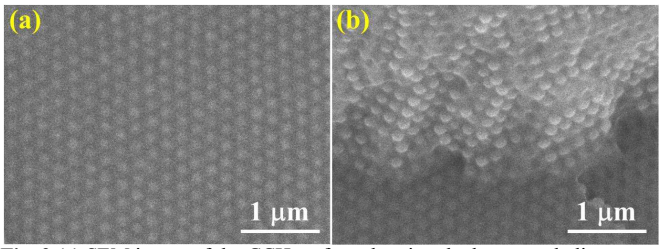


Fig. 2 (a) SEM image of the CCH surface showing the hexagonal alignment of the silica nanoparticles. (b) Image of the fracture section showing the ordered nanostructure in the inner structure.

The 3'- and 5'-amino-modified aptamers were then chemically coupled to the hydrogel network of the resultant CCH.[31] Before and after the aptamer functionalization, the reflection peak of CCH red shifted from 605 nm to 623nm. To investigate the specific of the aptamers to their corresponding targets, CCH with only A1 or A2 cross-linked to the network were prepared, respectively. These hydrogels were then incubated into solutions with only Hg^{2+} or Ag^{+}. Because of the specific binding between the aptamers and their corresponding targets, the blue-shift of the CCH was expected to be observed only when their corresponding targets were present. Fig. 3 was the results of the specific assay. It could be found that the diffraction colour of the hydrogel showed significant blue-shift only with their specific targets (Fig. S1). To further validate the specificity of the aptamer probes, non-target ions, such as Pb^{2+}, Cu^{2+}, Zn^{2+}, Mg^{2+}, Ca^{2+}, Al^{3+} and Fe^{3+} were used as the negative control inputs. It was found that only the solution of T1 or T2 could cause a significant diffraction shift, and the film displayed an undetectable optical response to the control metal ions (Table S2). Thus the aptamers cross-linked CCH showed restricted selectivity and specificity to their intended target, respectively.

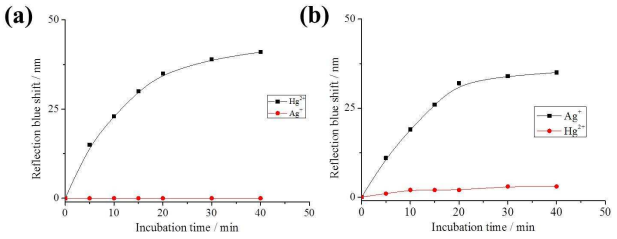


Fig. 3 (a) A1 hydrogel in the presence of Hg^{2+} and Ag^{+} and (b) A2 hydrogel in the presence of Hg^{2+} and Ag^{+}. The concentration of the targets was 0.10 μM.

After confirming the specific of the aptamers (A1 and A2) to their corresponding targets, the two aptamers were simultaneously coupled to the same hydrogel network of the CCH for the DNA logic gate fabrication. The logic shrinking performance of the aptamers functionalized CCH was firstly investigated by using target Hg^{2+} input. Usually, the shrinkage and the colour change of the CCH were influenced by the concentrations of the cross-linked aptamers and the target. To optimize these effects, we fixed the concentration of the A1 aptamer cross-linker in the CCH (0.2 mM) and analyzed the relationship between the diffracted colour and the concentrations of the target Hg^{2+}. It was found that the colour and the diffraction peak of the CCH shifted to blue gradually with the increasing of Hg^{2+} concentration, as shown in Fig. 4. Fig. S2 showed the relationship between the diffraction wavelength blue shift and the concentration of the Hg^{2+} concentration. As the CCH should undergo a visual logic colour change to achieve "OR" and "AND" logic performance, we chose 0.10 μM Hg^{2+} as the input signal T1, which could trigger an evident yellow diffraction colour in CCH.

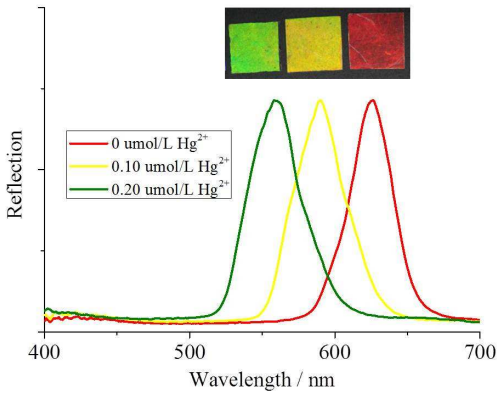


Fig. 4 The reflection spectra of the photonic hydrogel in the presence of different concentration of Hg^{2+}. Inset: diffraction color changes from red, yellow to green with increasing Hg^{2+} concentration.

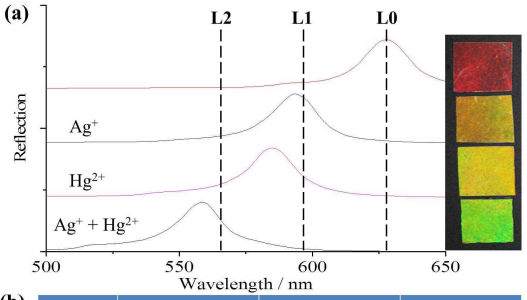
In order to obtain the similar shrink extent under the target Ag^{+} (T2) input, we fixed the concentration of T2 target the same as the T1 target (0.10 μM) and investigated the effect of the concentration of the A2 aptamer cross-linker on the structural colour blue shift. It was found that the diffraction peak was blue shifted with increase of

the A2 concentration and this shift reached 30 nm at A2 concentration of 0.5 mM. As the conformation change difference of A1 and A2, the shrinking extents of the two CCHs were different. Thus, the concentrations of 0.5 mM A2 and 0.2 mM A1 were used in the cross-linking reaction for the DNA CCH logic gate fabrication. Under this condition, the CCH showed yellow diffraction colour when exposed to T1 or T2 input and the logic "OR" gate could be defined.

The overall shrinkage and structural colour changes of the CCH upon the addition of T1 and T2 were following investigated. It was found that the shrinking extent of the CCH to T1 and T2 was nearly twice as high as the response of the CCH on exposure to T1 or T2. The reflection peak blue shifted to 560 nm by a complete conformation change of the A1 and A2 and the CCH showed brilliant green diffraction colour. Thus, the logic gate "AND" could be defined by using both T1 and T2.

To validate our design, the optimized aptamers cross-linked CCH was used as a basic DNA logic gate. Fig. 5a shows the results of our logic gate system under different inputs. It can be seen that the CCH DNA logic gate showed red colour (L0) in the absence of any input targets. The activation of the CCH with either T1 or T2 targets yielded yellow colour (L1). The incubation of the CCH with T1 and T2 obtained green colour with an obvious visible colour change (L2). If we defined the output signal was "1" when the blue-shift of the CCH reflection wavelength was greater than the threshold of 30 nm (L1) (Fig. S2). As discussed above, the true value of the CCH can be observed only by the presence of at least one of the targets and it is evident that T1 OR T2 was applied as an input. If the output signal "1" was defined when the blue-shift of the CCH reflection wavelength has reached or exceeded 60 nm (L2), which is possible only when T1 AND T2 exist simultaneously(Fig. S2). Thus, the choice of the gate type was realized by the choice of the signal level on the same CCH which eliminates the necessity of designing and using different types of materials (Fig. 5b).

(a)



(b)

	Input T1 Hg^{2+}	Input T2 Ag^+	Output
OR (L1)	0	0	0
	1	0	1
	0	1	1
	1	1	1

(c)

	Input T1 Hg^{2+}	Input T2 Ag^+	Output
AND (L2)	0	0	0
	1	0	0
	0	1	0
	1	1	1

Fig. 5 (a) Left: Reflection spectra of the CCH in the presence of Hg^{2+} and Ag^+ added separately or simultaneously. Right: Photograph showing the reflection blue shift for different combinations of two input signals. (b) The truth table of the logic "OR" when the choice of the signal level is L1. (c) The truth table of the logic "AND" when the choice of the signal level is L2.

Conclusions

In conclusion, we have incorporated colloidal crystal into hydrogel logic swelling system and developed an aptamer functionalized CCH that displayed colorimetric visual "OR" and "AND" functions. Taking advantage of the CCH's physicochemical behaviour, the specific aptamer-target recognition enables such CCH to undergo a colour change in response to the applied input stimuli and the outputs can be directly visualized by observing the diffraction colour of the CCH. In addition, the CCH could be designed to give "0" or "1" answer at two distinctly different signal colour levels: "OR" and "AND". This research opens up new opportunities toward colorimetric logic systems.

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Notes and references

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† Electronic supplementary information (ESI) available: I Experiment section. II Photograph of the aptamer functionalized CCH in present of different targets. III The specificity of the aptamer functionalized CCH. IV Relationship of the input ion concentration and the reflection wavelength blue shift. V The logic swelling kinetics of CCH. See DOI: 10.1039/c000000x/

1. C. Song, Z. G. Wang and B. Q. Ding, *Small*, 2013, **9**, 2382.
2. J. Yang, L. J. Shen, J. J. Ma, H. I. Schlaberg, S. Liu, J. Xu and C. Zhang, *ACS Appl. Mater. & Inter.*, 2013, **5**, 5392.
3. L. Zhang, Y. M. Zhang, R. P. Liang and J. D. Qiu, *J. Phys. Chem. C*, 2013, **117**, 12352.
4. K. S. Park, M. W. Seo, C. Jung, J. Y. Lee and H. G. Park, *Small*, 2012, **8**, 2203.
5. L. Wang, J. B. Zhu, L. Han, L. H. Jin, C. Z. Zhu, E. K. Wang and S. J. Dong, *Acs Nano*, 2012, **6**, 6659.
6. J. H. Chen, Z. Y. Fang, P. C. Lie and L. W. Zeng, *Anal. Chem.*, 2012, **84**, 6321.
7. H. Pei, L. Liang, G. B. Yao, J. Li, Q. Huang and C. H. Fan, *Angew Chem. Int. Edit.*, 2012, **51**, 9020.
8. Y. C. Dong, Z. Q. Yang and D. S. Liu, *Acc. Chem. Res.*, 2014, **47**, 1853.
9. Y. Q. Wen, L. P. Xu, C. B. Li, H. W. Du, L. F. Chen, B. Su, Z. L. Zhang, X. J. Zhang and Y. L. Song, *Chem. Commun.*, 2012, **48**, 8410.
10. K. Zhu, J. Z. Shen, R. Dietrich, A. Didier, X. Y. Jiang and E. Martlbauer, *Chem. Commun.*, 2014, **50**, 676.
11. M. I. Shukoor, M. O. Altman, D. Han, A. T. Bayrac, I. Ocsoy, Z. Zhu and W. H. Tan, *ACS Appl. Mater. & Inter.*, 2012, **4**, 3007.

12. T. Li, D. Ackermann, A. M. Hall and M. Famulok, *J. Am. Chem. Soc.*, 2012, **134**, 3508.
13. Y. L. Xianyu, K. Zhu, W. W. Chen, X. F. Wang, H. M. Zhao, J. S. Sun, Z. Wang and X. Y. Jiang, *Anal. Chem.*, 2013, **85**, 7029.
14. M. Zhou and S. J. Dong, *Acc. Chem. Res.*, 2011, **44**, 1232.
15. M. Zhou, Y. Du, C. G. Chen, B. L. Li, D. Wen, S. J. Dong and E. K. Wang, *J. Am. Chem. Soc.*, 2010, **132**, 2172.
16. X. M. Li, L. Sun and T. R. Ding, *Biosens. Bioelectron.*, 2011, **26**, 3570.
17. B. C. Yin, B. C. Ye, H. Wang, Z. Zhu and W. H. Tan, *Chem. Commun.*, 2012, **48**, 1248.
18. M. Gao, K. Gawel and B. T. Stokke, *Soft Matter.*, 2011, **7**, 1741.
19. E. Mastronardi, A. Foster, X. R. Zhang and M. C. DeRosa, *Sensors-Basel*, 2014, **14**, 3156.
20. M. Ikeda, T. Tanida, T. Yoshii, K. Kurotani, S. Onogi, K. Urayama and I. Hamachi, *Nat. Chem.*, 2014, **6**, 511.
21. K. Gawel and B. T. Stokke, *Soft Matter.*, 2011, **7**, 4615.
22. M. Z. Li, F. He, Q. Liao, J. Liu, L. Xu, L. Jiang, Y. L. Song, S. Wang and D. B. Zhu, *Angew Chem. Int. Edit.*, 2008, **47**, 7258.
23. Y. Huang, J. M. Zhou, B. Su, L. Shi, J. X. Wang, S. R. Chen, L. B. Wang, J. Zi, Y. L. Song and L. Jiang, *J. Am. Chem. Soc.*, 2012, **134**, 17053.
24. D. Y. Wang, J. S. Li, C. T. Chan, V. Sagueirino-Maceira, L. M. Liz-Marzan, S. Romanov and F. Caruso, *Small*, 2005, **1**, 122.
25. J. F. Galisteo-Lopez, M. Ibisate, R. Sapienza, L. S. Froufe-Perez, A. Blanco and C. Lopez, *Adv. Mater.*, 2011, **23**, 30.
26. D. M. Kuncicky, B. G. Prevo and O. D. Velev, *J. Mater. Chem.*, 2006, **16**, 1207.
27. J. H. Holtz and S. A. Asher, *Nature*, 1997, **389**, 829.
28. M. M. W. Muscatello, L. E. Stunja and S. A. Asher, *Anal. Chem.*, 2009, **81**, 4978.
29. B. F. Ye, F. Rong, H. C. Gu, Z. Y. Xie, Y. Cheng, Y. J. Zhao and Z. Z. Gu, *Chem. Commun.*, 2013, **49**, 5331.
30. B. F. Ye, H. B. Ding, Y. Cheng, H. C. Gu, Y. J. Zhao, Z. Y. Xie and Z. Z. Gu, *Adv. Mater.*, 2014, **26**, 3270.
31. B. F. Ye, Y. J. Zhao, Y. Cheng, T. T. Li, Z. Y. Xie, X. W. Zhao and Z. Z. Gu, *Nanoscale*, 2012, **4**, 5998.
32. J. P. Ge and Y. D. Yin, *Angew Chem. Int. Edit.*, 2011, **50**, 1492.
33. Y. J. Zhao, Z. Y. Xie, H. C. Gu, C. Zhu and Z. Z. Gu, *Chem. Soc. Rev.*, 2012, **41**, 3297.
34. N. Dave, M. Y. Chan, P. J. J. Huang, B. D. Smith and J. W. Liu, *J. Am. Chem. Soc.*, 2010, **132**, 12668.
35. J. W. Liu, *Soft Matter*, 2011, **7**, 6757.

