

CrossMark  
click for updatesCite this: *RSC Adv.*, 2017, 7, 1313

## A novel H<sub>2</sub>O<sub>2</sub> responsive supramolecular hydrogel for controllable drug release†

Chunhua Ren, Liping Chu, Fan Huang, Lijun Yang, Huirong Fan,\* Jianfeng Liu and Cuihong Yang\*

Due to the important significance of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in physiology, aging and disease in living organisms, tremendous effort has been devoted to develop H<sub>2</sub>O<sub>2</sub> responsive materials for the detection of its over production or for controlled drug release. However, it is still challenging to develop H<sub>2</sub>O<sub>2</sub> responsive supramolecular hydrogels. In this study, we designed and synthesized a novel H<sub>2</sub>O<sub>2</sub> responsive peptide hydrogelator bearing the thiazolidinone group. A supramolecular hydrogel based on peptide self-assembly was prepared through a heating-cooling process and its gel-sol phase transition could be triggered by the removal of thiazolidinone groups upon H<sub>2</sub>O<sub>2</sub> oxidation. The excellent H<sub>2</sub>O<sub>2</sub> responsive property of the supramolecular hydrogel was investigated by LC-MS, rheology and TEM. A drug release study *in vitro* demonstrated that the gel-sol phase transition could be applied for releasing gemcitabine sustainedly and controllably. Our study could provide a new way for the design of H<sub>2</sub>O<sub>2</sub> responsive materials and hold great potential in the application of anticancer drug delivery.

Received 9th November 2016  
Accepted 14th December 2016

DOI: 10.1039/c6ra26536g

www.rsc.org/advances

Peptide-based supramolecular hydrogels have attracted extensive research interest in the past few decades due to their inherent advantages, such as ease of design and synthesis, good biocompatibility and degradability.<sup>1</sup> Since supramolecular hydrogels are formed by non-covalent interactions (hydrogen bond,  $\pi$ - $\pi$ , hydrophobic, and charge interactions), they are therefore extremely sensitive to external stimuli, including pH,<sup>2</sup> light,<sup>3</sup> enzymes,<sup>4</sup> ions,<sup>5</sup> and redox agents.<sup>6</sup> Increasing numbers of intelligent responsive supramolecular hydrogels have been developed so far, and they have shown great promise in the applications of drug delivery,<sup>7</sup> cancer cell inhibition,<sup>8</sup> vaccine adjuvants<sup>9</sup> and detection of important analytes.<sup>10</sup>

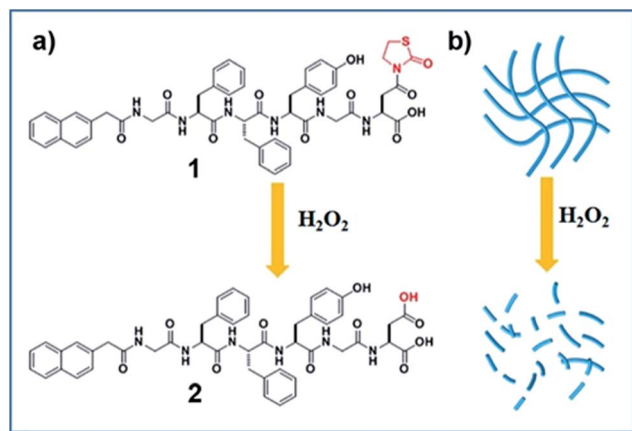
Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), as a second messenger for intracellular signal transduction,<sup>11</sup> usually results from cellular metabolism of molecular oxygen. In most cases, it is maintained at well-balanced concentration levels and plays crucial roles in cell proliferation, cell differentiation and cell migration.<sup>12</sup> The over-production of H<sub>2</sub>O<sub>2</sub> can lead to high oxidative stress and therefore impaired cellular structures.<sup>13</sup> Many reports have demonstrated that a series of pathologies are associated with elevated levels of H<sub>2</sub>O<sub>2</sub> including inflammation,<sup>14</sup> cancer,<sup>15</sup> cardiovascular disorders,<sup>16</sup> and neurodegenerative diseases.<sup>17</sup> Consequently, enormous efforts have been made to develop H<sub>2</sub>O<sub>2</sub> responsive nano-systems for controlled

drug release to treat these diseases or for the detection of its over-production. These nano-systems are mostly based on the conventional peroxalate ester that can react with H<sub>2</sub>O<sub>2</sub>. For example, Murthy and co-workers have reported on nanoparticles formulated from peroxalate and fluorescent dyes capable of imaging hydrogen peroxide *in vivo* with high specificity and sensitivity.<sup>18</sup> There are also several reports regarding H<sub>2</sub>O<sub>2</sub> responsive supramolecular hydrogels. For example, Hamachi and co-workers have recently reported on responsive peptide-based hydrogels containing a H<sub>2</sub>O<sub>2</sub>-reactive boronate-ylmethoxycarbonyl group and capable of retaining the activity of encapsulated enzymes.<sup>19</sup> They have demonstrated that the programmable hybridization of the hydrogel and oxidases enables the resulting materials responding to not only H<sub>2</sub>O<sub>2</sub> molecules but also a variety of disease-related biomarkers. These pioneering works highlight the importance of H<sub>2</sub>O<sub>2</sub> responsive materials including the supramolecular hydrogels.

Recently, a novel H<sub>2</sub>O<sub>2</sub> responsive group, thiazolidinone modified carboxylic acid, has been reported, and it can release free carboxylic acid upon the activation of H<sub>2</sub>O<sub>2</sub>.<sup>20</sup> Inspired by this work, we opted to develop a novel H<sub>2</sub>O<sub>2</sub> responsive supramolecular hydrogelator bearing the thiazolidinone group. We therefore designed a short peptide derivative Nap-GFFYGD(Thi) (compound 1) bearing a H<sub>2</sub>O<sub>2</sub> responsive thiazolidinone at the C-terminal of the peptide. As shown in Scheme 1, compound 1 was expected to form nanofibers and a hydrogel by supramolecular self-assembly at a given concentration. The removal of thiazolidinone through the oxidation/elimination reaction by H<sub>2</sub>O<sub>2</sub> was expected to produce a more hydrophilic peptide Nap-GFFYGD (2), resulting the dis-assembly of nanofibres and a gel-

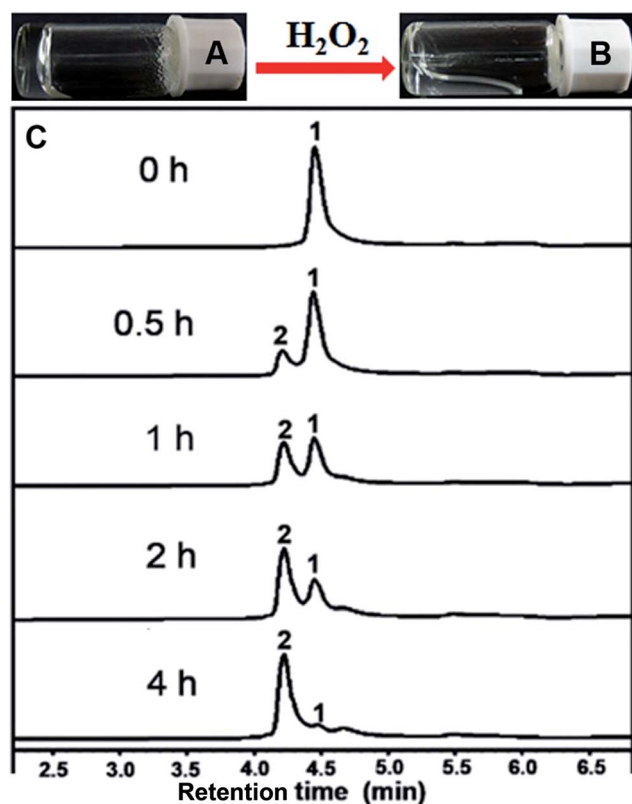
Tianjin Key Laboratory of Radiation Medicine and Molecular Nuclear Medicine, Institute of Radiation Medicine, Chinese Academy of Medical Science and Peking Union Medical College, Tianjin, 300192, China. E-mail: yangcuihong@irm-cams.ac.cn

† Electronic supplementary information (ESI) available: Synthesis and characterization, hydrogel formation, determination of conversion percentage, rheological data. See DOI: 10.1039/c6ra26536g



**Scheme 1** (a) Chemical structures of Nap-GFFYGD(Thi) (compound 1) and Nap-GFFYGD (compound 2) and  $\text{H}_2\text{O}_2$  triggered conversion. (b) Schematic representation of possible  $\text{H}_2\text{O}_2$  triggered hydrogel degradation.

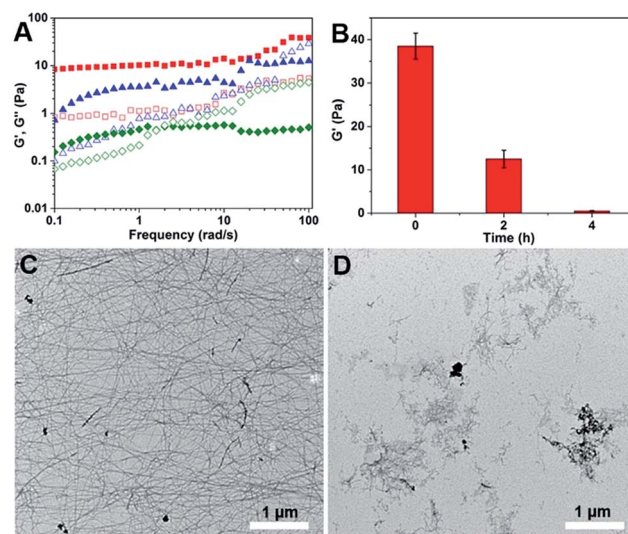
sol transition. The synthesis route and purification process of Fmoc-aspartic acid decorated with thiazolidinone (D(Thi)) were described in Scheme S1.<sup>†</sup> We then prepared compound 1 by standard solid phase peptide synthesis (SPPS) and obtained the pure compound by reverse-phase high performance liquid chromatography (HPLC).



**Fig. 1** Optical images of the hydrogel of compound 1 (0.1 wt%) in PBS solution (A) and the resulting solution upon adding 40 mM  $\text{H}_2\text{O}_2$  to the gel at 37 °C for 4 hours (B) and (C) HPLC traces to demonstrate the transformation triggered by 40 mM  $\text{H}_2\text{O}_2$  at 37 °C within 4 hours.

After obtaining the designed compound, the gelation property of compound 1 was firstly examined. Results in Fig. 1A indicated that it could form a hydrogel at a minimum gelation concentration (MGC) of 0.1 wt% within 5 minutes upon a heating-cooling process. We then tested the  $\text{H}_2\text{O}_2$  responsive property of the hydrogel. A gel-sol transformation was observed after adding  $\text{H}_2\text{O}_2$  (40 mM) to the gel at 37 °C for 4 hours (Fig. 1B).  $\text{H}_2\text{O}_2$ -response sensitivity of the hydrogel was evaluated through the gel-sol transition after the addition of 0–100 mM  $\text{H}_2\text{O}_2$  to the gel. The results showed that at least 4 mM  $\text{H}_2\text{O}_2$  was required to induce a complete collapse of the gel after 24 hours (Fig. S9<sup>†</sup>) and the time required for the gel-sol transition ranged from 1.5 to 24 hours depending on the amount of  $\text{H}_2\text{O}_2$  (Fig. S10<sup>†</sup>). Liquid chromatography mass spectrometer (LC-MS) was employed to analyse the gel-sol phase transition. As shown in Fig. 1C, compound 1 gradually converted to compound 2 after the addition of  $\text{H}_2\text{O}_2$ . For instance, the conversion percentage was about 50% at one hour time point and reached an equilibrium after about four hours with a conversion rate of about 96% (Fig. S11<sup>†</sup>). The mass spectra of resulting solution in Fig. 1B (Fig. S7<sup>†</sup>) indicated the molecular weight peak of Nap-GFFYGD (2). These observations clearly demonstrated the success of our design, and the  $\text{H}_2\text{O}_2$  responsive property of our hydrogel suggested its potential application in controlled drug release.

Rheology was also performed to characterize the mechanical properties of the hydrogels before and after the addition of  $\text{H}_2\text{O}_2$ . The dynamic frequency sweep was carried at a fixed strain value of 0.5% in the frequency range from 0.1 to 100  $\text{rad s}^{-1}$ . As shown in Fig. 2A and S12,<sup>†</sup> before the addition of  $\text{H}_2\text{O}_2$ , the  $G'$  value of the hydrogel was an order of magnitude bigger than its



**Fig. 2** (A) Dynamic frequency sweep at the strain of 0.5% after the addition of  $\text{H}_2\text{O}_2$  at different time points (solid symbols,  $G'$ ; hollow symbols,  $G''$ ): 0 h (squares), 2 h (triangles) and 4 h (diamond), (B) the  $G'$  value at the mode of dynamic frequency sweep at the frequency of 10  $\text{rad s}^{-1}$  and strain of 0.5% after the addition of  $\text{H}_2\text{O}_2$  at different time points. TEM images of the gel without  $\text{H}_2\text{O}_2$  (C) and with 40 mM  $\text{H}_2\text{O}_2$  overnight (D).



$G''$  value in the tested frequency range, indicating a true hydrogel formation.<sup>21</sup> After incubation with  $H_2O_2$  for two hours and four hours, the  $G''$  value of the samples become bigger than their corresponding  $G'$  value at frequency value of 50 and 2 rad  $s^{-1}$ , respectively. At the same time, the  $G'$  value decreased dramatically from 40 Pa to 0.4 Pa after four hours' incubation with  $H_2O_2$  (Fig. 2B). These observations suggested the formation of viscous solutions after the addition of  $H_2O_2$ . Transmission electron microscopy (TEM) was then used to characterize the morphology of nanostructures in the hydrogel and the obtained solution. As shown in Fig. 2C, uniform nanofibers were observed in the hydrogel with the diameter of about 25 nm and the length of up to several microns. They entangled with each other to form dense networks for the hydrogel formations. Upon the addition of  $H_2O_2$  overnight, the long nanofibers disappeared and only few short nanofibers could be observed (Fig. 2D).

We therefore tested the possible application of our responsive hydrogel in controlled drug release. We choose gemcitabine as a drug molecule to investigate its controlled release property from our hydrogel at 37 °C. A 0.25 mL of PBS solution with different amounts of  $H_2O_2$  was placed on top of the gel (0.2 mL). The upper solution was totally taken out at different time intervals following by adding another 0.25 mL fresh PBS solution containing corresponding  $H_2O_2$ . The accumulation percentage of released gemcitabine from the hydrogel were quantified by LC-MS based on a standard curve. Results in Fig. 3 demonstrated that the release speed of gemcitabine depended on the amount of  $H_2O_2$  in PBS solution. That was, the more  $H_2O_2$  in PBS solution, the faster gemcitabine released from the hydrogel. In the absence of  $H_2O_2$ , the accumulative release percentage of gemcitabine was only about 10% in 12 hours. While in the presence of 4 and 10 mM  $H_2O_2$ , the accumulative release percentage of gemcitabine from the gel was about 30% and 50%, respectively in 12 hours. Since cancer cells exhibit elevated levels of  $H_2O_2$  compared with normal cells,<sup>22</sup> our  $H_2O_2$  responsive peptide

hydrogel might be used as a potential delivery system for anticancer drug delivery to tumor sites.

In summary, we have developed a novel supramolecular hydrogel based on peptide self-assembly with a gel-sol phase transition triggered by  $H_2O_2$ . The hydrogel showed an excellent  $H_2O_2$  responsive property and could release gemcitabine sustainedly and controllably. Compared with conventional  $H_2O_2$  responsive materials involving peroxalate ester or boronoaryl groups, the synthesis of thiazolidinone modified hydrogelator were relatively easier and more straightforward, and the gelator could avoid being cleaved by ubiquitous esterases *in vivo*. However, due to the limited structure change of thiazolidinone modified hydrogelator upon  $H_2O_2$  oxidization, the  $H_2O_2$ -response sensitivity of the hydrogelator developed by us was not as high as that of peroxalate ester and boronoaryl groups and should be further improved in the future study. As the most important marker for reactive oxygen species (ROS),  $H_2O_2$  is increasingly investigated in physiology, aging and disease in living organisms. Our supramolecular hydrogel system could provide a new way for the detection of the over-produced  $H_2O_2$  and hold great potential in the application of anticancer drug delivery.

## Acknowledgements

This work is supported by National Natural Science Foundation of China (No. 81471727), Outstanding Young Faculty Award of Peking Union Medical College (YR1579), PUMC Youth Fund and the Fundamental Research Funds for the Central Universities (3332015100), Fundamental Research Funds for CAMS & PUMC (2016ZX310082) and CAMS Initiative for Innovative Medicine (2016-12M-3-022).

## Notes and references

- 1 J. H. Collier, J. S. Rudra, J. Z. Gasiorowski and J. P. Jung, *Chem. Soc. Rev.*, 2010, **39**, 3413–3424; S. Toledano, R. J. Williams, V. Jayawarna and R. V. Ulijn, *J. Am. Chem. Soc.*, 2006, **128**, 1070–1071; X. Zhao and S. Zhang, *Chem. Soc. Rev.*, 2006, **35**, 1105–1110; S. Fleming and R. V. Ulijn, *Chem. Soc. Rev.*, 2014, **43**, 8150–8177; J. W. Steed, *Chem. Commun.*, 2011, **47**, 1379–1383; S. S. Babu, V. K. Praveen and A. Ajayaghosh, *Chem. Rev.*, 2014, **114**, 1973–2129; J. Raeburn, A. Z. Cardoso and D. J. Adams, *Chem. Soc. Rev.*, 2013, **42**, 5143–5156.
- 2 K. L. Morris, L. Chen, J. Raeburn, O. R. Sellick, P. Cotanda, A. Paul, P. C. Griffiths, S. M. King, R. K. O'Reilly, L. C. Serpell and D. J. Adams, *Nat. Commun.*, 2013, **4**, 1480; D. J. Cornwell, B. O. Okesola and D. K. Smith, *Angew. Chem., Int. Ed.*, 2014, **126**, 12669–12673; D. J. Cornwell, O. J. Daubney and D. K. Smith, *J. Am. Chem. Soc.*, 2015, **137**, 15486–15492; Z. Sun, Z. Li, Y. He, R. Shen, L. Deng, M. Yang, Y. Liang and Y. Zhang, *J. Am. Chem. Soc.*, 2013, **135**, 13379–13386.
- 3 T. Yoshii, M. Ikeda and I. Hamachi, *Angew. Chem., Int. Ed.*, 2014, **126**, 7392–7395; J. Li, J. Carnall, M. C. Stuart and S. Otto, *Angew. Chem., Int. Ed.*, 2011, **50**, 8384–8386;

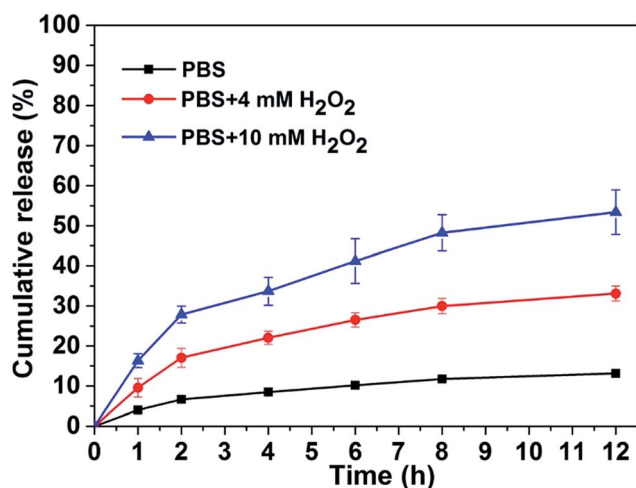


Fig. 3 Release profile of gemcitabine from the hydrogel with different amounts of  $H_2O_2$ .





- A. Gopal, M. Hifsudheen, S. Furumi, M. Takeuchi and A. Ajayaghosh, *Angew. Chem., Int. Ed.*, 2012, **124**, 10657–10661; M. He, J. Li, S. Tan, R. Wang and Y. Zhang, *J. Am. Chem. Soc.*, 2013, **135**, 18718–18721; B. Xue, Y. Li, F. Yang, C. Zhang, M. Qin, Y. Cao and W. Wang, *Nanoscale*, 2014, **6**, 7832–7837.
- 4 A. R. Hirst, S. Roy, M. Arora, A. K. Das, N. Hodson, P. Murray, S. Marshall, N. Javid, J. Sefcik, J. Boekhoven, J. H. Van Esch, S. Santabarbara, N. T. Hunt and R. V. Uljin, *Nat. Chem.*, 2010, **2**, 1089–1094; C. G. Pappas, I. R. Sasselli and R. V. Uljin, *Angew. Chem., Int. Ed.*, 2015, **127**, 8237–8241; S. K. M. Nalluri, C. Berdugo, N. Javid, P. W. Frederix and R. V. Uljin, *Angew. Chem., Int. Ed.*, 2014, **126**, 5992–5997; J. Zhou, X. Du, J. Li, N. Yamagata and B. Xu, *J. Am. Chem. Soc.*, 2015, **137**, 10040–10043; J. Nanda, A. Biswas, B. Adhikari and A. Banerjee, *Angew. Chem., Int. Ed.*, 2013, **52**, 5041–5045; A. K. Das, I. Maity, H. S. Parmar, T. O. McDonald and M. Konda, *Biomacromolecules*, 2015, **16**, 1157–1168.
- 5 W. Edwards and D. K. Smith, *J. Am. Chem. Soc.*, 2014, **136**, 1116–1124; C. M. Micklitsch, P. J. Knerr, M. C. Branco, R. Nagarkar, D. J. Pochan and J. P. Schneider, *Angew. Chem., Int. Ed.*, 2011, **123**, 1615–1617; M.-O. M. Piepenbrock, G. O. Lloyd, N. Clarke and J. W. Steed, *Chem. Rev.*, 2009, **110**, 1960–2004; J. W. Steed, *Chem. Soc. Rev.*, 2010, **39**, 3686–3699; J. S. Foster, J. M. Žurek, N. M. Almeida, W. E. Hendriksen, V. A. le Sage, V. Lakshminarayanan, A. L. Thompson, R. Banerjee, R. Eelkema and H. Mulvana, *J. Am. Chem. Soc.*, 2015, **137**, 14236–14239; Z. Shen, Y. Jiang, T. Wang and M. Liu, *J. Am. Chem. Soc.*, 2015, **137**, 16109–16115; J. Boekhoven, W. E. Hendriksen, G. J. Koper, R. Eelkema and J. H. van Esch, *Science*, 2015, **349**, 1075–1079.
- 6 C. J. Bowerman and B. L. Nilsson, *J. Am. Chem. Soc.*, 2010, **132**, 9526–9527; C. Ren, Z. Song, W. Zheng, X. Chen, L. Wang, D. Kong and Z. Yang, *Chem. Commun.*, 2011, **47**, 1619–1621; X. Miao, W. Cao, W. Zheng, J. Wang, X. Zhang, J. Gao, C. Yang, D. Kong, H. Xu, L. Wang and Z. Yang, *Angew. Chem., Int. Ed.*, 2013, **52**, 7781–7785; Y. Zhang, B. Zhang, Y. Kuang, Y. Gao, J. Shi, X. X. Zhang and B. Xu, *J. Am. Chem. Soc.*, 2013, **135**, 5008–5011.
- 7 S. Chen, L. Rong, Q. Lei, P. Cao, S. Qin, D. Zheng, H. Jia, J. Zhu, S. Cheng, R. Zhuo and X. Zhang, *Biomaterials*, 2016, **77**, 149–163; J. Liu, J. Liu, L. Chu, Y. Zhang, H. Xu, D. Kong, Z. Yang, C. Yang and D. Ding, *ACS Appl. Mater. Interfaces*, 2014, **6**, 5558–5565; S. Qin, M. Peng, L. Rong, B. Li, S. Wang, S. Cheng, R. Zhuo and X. Zhang, *Regener. Biomater.*, 2015, **2**, 159–166; T. Su, Z. Tang, H. He, W. Li, X. Wang, C. Liao, Y. Sun and Q. Wang, *Chem. Sci.*, 2014, **5**, 4204–4209; D. Das, T. Kar and P. K. Das, *Soft Matter*, 2012, **8**, 2348–2365; Y. Yuan, L. Wang, W. Du, Z. Ding, J. Zhang, T. Han, H. Zhang and G. Ling, *Angew. Chem., Int. Ed.*, 2015, **54**, 9700–9704.
- 8 R. A. Pires, Y. M. Abul-Haija, D. S. Costa, R. Novoa-Carballal, R. L. Reis, R. V. Uljin and I. Pashkuleva, *J. Am. Chem. Soc.*, 2015, **137**, 576–579; Y. Kuang, J. Shi, J. Li, D. Yuan, K. A. Alberti, Q. Xu and B. Xu, *Angew. Chem., Int. Ed.*, 2014, **53**, 8104–8107; J. Li, Y. Kuang, J. Shi, J. Zhou, J. E. Medina, R. Zhou, D. Yuan, C. Yang, H. Wang, Z. Yang, J. Liu, D. Dinulescu and B. Xu, *Angew. Chem., Int. Ed.*, 2015, **127**, 13505–13509; H. Wang, Z. Feng, D. Wu, K. J. Fritzsche, M. Rigney, J. Zhou, Y. Jiang, K. Schmidt-Rohr and B. Xu, *J. Am. Chem. Soc.*, 2016, **138**, 10758–10761; Z. Zheng, P. Chen, M. Xie, C. Wu, Y. Luo, W. Wang, J. Jiang and G. Liang, *J. Am. Chem. Soc.*, 2016, **138**, 11128–11131.
- 9 J. S. Rudra, T. Sun, K. C. Bird, M. D. Daniels, J. Z. Gasiowski, A. S. Chong and J. H. Collier, *ACS Nano*, 2012, **6**, 1557–1564; J. S. Rudra, S. Mishra, A. S. Chong, R. A. Mitchell, E. H. Nardin, V. Nussenzweig and J. H. Collier, *Biomaterials*, 2012, **33**, 6476–6484; J. Chen, R. R. Pompano, F. W. Santiago, L. Maillat, R. Sciammas, T. Sun, H. Han, D. J. Topham, A. S. Chong and J. H. Collier, *Biomaterials*, 2013, **34**, 8776–8785; G. A. Hudalla, T. Sun, J. Z. Gasiowski, H. Han, Y. F. Tian, A. S. Chong and J. H. Collier, *Nat. Mater.*, 2014, **13**, 829–836; Y. Tian, H. Wang, Y. Liu, L. Mao, W. Chen, Z. Zhu, W. Liu, W. Zheng, Y. Zhao and D. Kong, *Nano Lett.*, 2014, **14**, 1439–1445; H. Wang, Z. Luo, Y. Wang, T. He, C. Yang, C. Ren, L. Ma, C. Gong, X. Li and Z. Yang, *Adv. Funct. Mater.*, 2016, **26**, 1822–1829.
- 10 C. Ren, H. Wang, D. Mao, X. Zhang, Q. Fengzhao, Y. Shi, D. Ding, D. Kong, L. Wang and Z. Yang, *Angew. Chem., Int. Ed.*, 2015, **54**, 4823–4827; C. Ren, J. Zhang, M. Chen and Z. Yang, *Chem. Soc. Rev.*, 2014, **43**, 7257–7266; R. Peltier, G. Chen, H. Lei, M. Zhang, L. Gao, S. S. Lee, Z. Wang and H. Sun, *Chem. Commun.*, 2015, **51**, 17273–17276; Y. Cai, J. Zhan, H. Shen, D. Mao, S. Ji, R. Liu, B. Yang, D. Kong, L. Wang and Z. Yang, *Anal. Chem.*, 2015, **88**, 740–745; T. Xu, C. Liang, S. Ji, D. Ding, D. Kong, L. Wang and Z. Yang, *Anal. Chem.*, 2016, **88**, 7318–7323; L. L. Lock, C. D. Reyes, P. Zhang and H. Cui, *J. Am. Chem. Soc.*, 2016, **138**, 3533–3540.
- 11 S. G. Rhee, *Science*, 2006, **312**, 1882–1883.
- 12 J. Fang, T. Seki and H. Maeda, *Adv. Drug Delivery Rev.*, 2009, **61**, 290–302; G. Groeger, C. Quiney and T. G. Cotter, *Antioxid. Redox Signaling*, 2009, **11**, 2655–2671.
- 13 B. C. Dickinson and C. J. Chang, *J. Am. Chem. Soc.*, 2008, **130**, 9638–9639.
- 14 J. Kwon, J. Kim, S. Park, G. Khang, P. M. Kang and D. Lee, *Biomacromolecules*, 2013, **14**, 1618–1626.
- 15 E. O. Hileman, J. Liu, M. Albitar, M. J. Keating and P. Huang, *Cancer Chemother. Pharmacol.*, 2004, **53**, 209–219; S. Kawanishi, Y. Hiraku, S. Pinlaor and N. Ma, *J. Biol. Chem.*, 2006, **387**, 365–372.
- 16 K. Sugamura and J. F. Keaney, *Free Radical Biol. Med.*, 2011, **51**, 978–992.
- 17 K. J. Barnham, C. L. Masters and A. I. Bush, *Nat. Rev. Drug Discovery*, 2004, **3**, 205–214.
- 18 D. Lee, S. Khaja, J. C. Velasquez-Castano, M. Dasari, C. Sun, J. Petros, W. R. Taylor and N. Murthy, *Nat. Mater.*, 2007, **6**, 765–769.
- 19 M. Ikeda, T. Tanida, T. Yoshii, K. Kurotani, S. Onogi, K. Urayama and I. Hamachi, *Nat. Chem.*, 2014, **6**, 511–518;



- T. Yoshii, S. Onogi, H. Shigemitsu and I. Hamachi, *J. Am. Chem. Soc.*, 2015, **137**, 3360–3365.
- 20 C. Perez, J.-P. Monserrat, Y. Chen and S. M. Cohen, *Chem. Commun.*, 2015, **51**, 7116–7119.
- 21 J. Raeburn, G. Pont, L. Chen, Y. Cesbron, R. Lévy and D. J. Adams, *Soft Matter*, 2012, **8**, 1168–1174.
- 22 R. Kumar, J. Han, H. J. Lim, W. Ren, J. Y. Lim, J. H. Kim and J. S. Kim, *J. Am. Chem. Soc.*, 2014, **136**, 17836–17843.

