Organic & Biomolecular Chemistry

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Org. Biomol. Chem.*, 2014, **12**, 1383

Received 18th October 2013,
Accepted 7th January 2014

DOI: 10.1039/c3ob42078g

www.rsc.org/obc

Enzyme-controllable F-NMR turn on through disassembly of peptide-based nanospheres for enzyme detection†

Jie Gao,[a] Yang Shi,[a] Youzhi Wang,[a] Yanbin Cai,[a] Jie Shen,*[b] Deling Kong[a,c] and Zhimou Yang*[a]

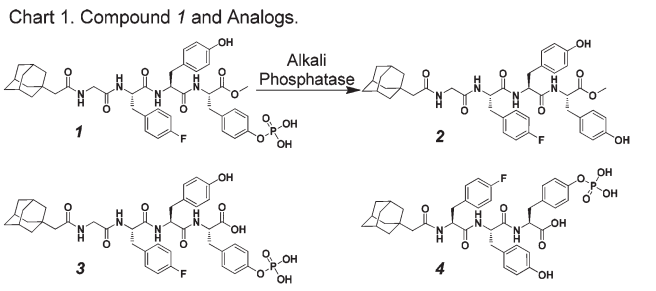
The enzyme tyrosinase could trigger the disassembly of peptide-based nanospheres, resulting in F-NMR signal turning on.

Self-assembling peptide-based nano-materials have shown great potential in both fields of biomedicine and materials science. They have been applied to drug delivery,[1,2] imaging of important analysts,[3] regenerative medicine,[4] synthesis of organic and inorganic materials,[5,6] *etc.* Among these systems, responsive peptide-based assembling systems are probably the most extensively investigated ones because they are very useful for many applications including drug delivery on demand and screening many important analysts.[5,7] For instance, self-assembling nanofibers of drug–peptide conjugates can constantly release therapeutic agents in acidic or reductive environments.[2,8] The sol–gel phase transitions have also been utilized to detect enzymes and peroxide-based explosives.[9,10] Recently, Hamachi and co-workers reported that the F-NMR signal was silent for fluoride (F)-containing compounds in the self-assembled state,[11] and disassembly of these compounds through specific ligand–receptor interactions could lead to F-NMR signal turning on. This self-assembling and disassembling process could be developed into a general strategy for detection of many important biomacromolecules. Stimulated by these pioneering studies, we have reported in this study F-containing short peptide-based nanospheres that could respond to the enzyme tyrosinase for its detection.

We had found that a phosphorylated short peptide capped with the adamantane (Ada) group, Ada-GFFpY-OMe, could form nanospheres upon enzymatic triggering.[12] We had also reported on a molecular gelator (Ac-YYYY-OMe) that could show a gel–sol phase transition to the enzyme tyrosinase because the enzyme could oxidize phenol on Y to quinone with much less π–π stacking ability.[10] Based on this information and the aforementioned result reported by Hamachi *et al.*, we opted to develop a self-assembled nanosphere system based on a F-containing short peptide that could respond to the enzyme tyrosinase. The enzyme tyrosinase was overexpressed in melanoma and we believed that such a responsive nanosphere system might be used for the detection of melanoma by F-NMR signal turn on.

We therefore designed and synthesized three phosphorylated short peptides with Ada-capping groups, Ada-GfFYpY-OMe (**1** in Scheme 1), Ada-GfFYpY-OH (**3** in Scheme 1), and Ada-fFYpY-OH (**4** in Scheme 1). These peptides might self-assemble into nanospheres upon treatment with phosphatase. If so, the F-NMR signal from fluoride-containing phenylalanine (fF) should be in silence due to the formation of nanospheres (Scheme 2). Treating the nanospheres with tyrosinase might lead to their disassembly into homogeneous solutions, thus restoring the F-NMR signal (Scheme 2). This process could therefore be applied to the detection of tyrosinase.

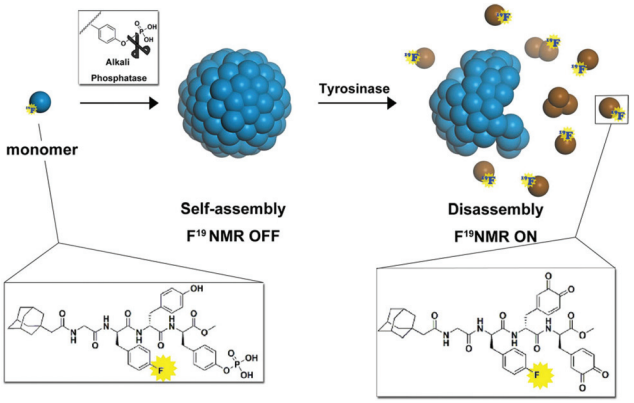
We firstly studied the self-assembly property of the three peptides upon treatment with phosphatase. As shown in Fig. 1, Ada-fFYpY-OH (compound **4**, Fig. 1B) and Ada-GfFYpY-OH (compound **3**, Fig. 1C) formed a precipitate and a clear



Scheme 1 Chemical structures of Ada-GfFYpY-OMe (**1**), Ada-GfFYpY-OH (**3**), and Ada-fFYpY-OH (**4**) and schematic illustration of phosphatase-triggered conversion from **1** to **2**.

[a]*State Key Laboratory of Medicinal Chemical Biology and College of Life Sciences, Nankai University, Tianjin 300071, P. R. China. E-mail: yangzm@nankai.edu.cn*
[b]*Tianjin First Center Hospital, Tianjin 300192, P. R. China. E-mail: shenjie_vip@126.com*
[c]*Tianjin Key Laboratory of Biomaterial Research, Institute of Biomedical Engineering, Chinese Academy of Medical Science, Tianjin 300192, P. R. China*

† Electronic supplementary information (ESI) available: Synthesis and characterization, nanosphere preparation, NMR spectra after adding enzymes, size distribution of nanospheres. See DOI: 10.1039/c3ob42078g



Scheme 2 Schematic illustration of enzymatic responding Off/On ^{19}F NMR probes.

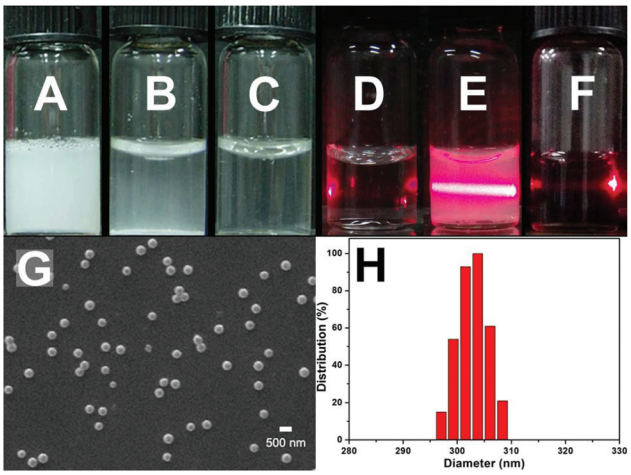


Fig. 1 Optical images of PBS solutions (pH 7.4) of (A) spheres, (B) precipitates, and (C) clear solution formed by adding phosphatase (25 U mL^{-1}) to solutions containing 0.15 wt% of Ada-GfFpYY-OMe (**1**), Ada-fFpYY-OMe (**4**), and Ada-GfFpYY-OH (**3**), respectively. (D) No visible beam occurred for the solution of **1**, (E) a visible beam occurred after the addition of phosphatase to the solution of **1**, (F) a very weak beam occurred 1 hour after the addition of tyrosinase (200 U mL^{-1}) to the suspension shown in (E). (G) SEM image of nanospheres in solution of (A), and (H) diameter distribution of spheres in solution of (A) detected by dynamic light scattering.

solution in phosphate buffer saline (PBS, pH = 7.4) with phosphatase, respectively (concentration of peptide was 0.15 wt% and concentration of enzyme was 25 U mL^{-1}). Only Ada-GfFYpY-OMe (compound **1**) could form a stable dispersion upon treatment with phosphatase (Fig. 1A). These observations indicated that the glycine (G) between Ada and the peptide and the terminal methyl ester were crucial to the formation of a stable dispersion. These phenomena were also consistent with our published results that Nap-GFFpY-OMe exhibited a better gelation ability than both Nap-FFpY-OMe and Nap-GFFpY-OH upon treatment with phosphatase.[13] The dispersion of Nap-GfFYpY-OMe with phosphatase in PBS was stable for up to one week (Fig. 1E). Since the nanospheres were composed of short peptides, they could be destroyed by adding large amounts of organic solvents such as DMSO (10% v/v), DMF (10% v/v) or THF (15% v/v), so their working environment must be in water or PBS.

We then used scanning electron microscopy (SEM) to characterize the morphology of self-assembled nanostructures in Fig. 1A. We observed uniform nanospheres with a diameter of about 300 nm in the sample (Fig. 1G). The dynamic light scattering (DLS) result showed the size distribution of the nanospheres (295–310 nm, Fig. 1H). Both results indicated a narrow size distribution of the nanospheres. The enzyme concentration had little effect while the concentration of peptide had a dramatic effect on the size of the resulting nanospheres. As shown in Table S-1,† for PBS solutions of **1** (0.05 wt%) treated with different concentrations of phosphatase, the size of the resulting nanospheres was similar (around 650 nm), while solutions with higher concentrations of **1** would lead to the formation of nanospheres with smaller sizes (Table S-2,† 246, 377, 482 and 684 nm from solutions of **1** at concentrations of 0.2, 0.1, 0.05, and 0.005 wt%, respectively). The reason why this would happen was complicated, and we came to a hypothesis that this relationship between concentration and diameter could be explained by the model of nucleation and crystal growth in crystallization, which was clearly explicated by reseachers.[14] Salts in dilute solution would often lead to larger crystals due to slow nucleation and better crystal growth. Similarly, lower concentrations of compound **1** might cause very slow sphere nuleation so that it could grow into larger particles. The concentration difference of phosphatase added to the solution might not be large enough to affect the nucleation, thus resulting in almost the same size of particles.

Similar to Hamachi's results, the F-NMR signal was silent in nanospheres. As shown in Fig. 2A (the top spectrum), we observed a single peak at −116.34 ppm from the F-NMR spectrum of **1** in D_2O (0.15 wt%, pH = 7.4). For the nanosphere dispersion (Fig. 2A, the spectrum in the bottom), we could not observe any signals. We then added the enzyme of tyrosinase (200 U mL^{-1}) to check whether the F-NMR signal could be restored or not. The results shown in Fig. 2B (peaks marked by round dots) indicated that a peak at −116.43 ppm appeared and its intensity increased gradually from 0 to 60 minutes. These observations suggested the disassembly of nanospheres due to the oxidation of phenol to diphenol with less self-assembly ability (SEM images in Fig. S-7,† ^1H NMR spectrum in Fig. S-8†). We observed a second signal at about 75 minute time point (Fig. 2C, peaks marked by triangle dots) probably due to the formation of quinone. The intensity of the peak at −116.43 ppm from diphenol decreased and that at −116.53 ppm from quinone increased gradually from 75 to 135 minute time point, indicating the transformation from diphenol to quinone (^1H NMR spectrum in Fig. S-9†). The F-NMR signal turn on suggested the potential of our nanosphere system for the detection of tyrosinase.

We also tested the signal restoring ability of the nanospheres responding to various concentrations of tyrosinase (Fig. 2D). In the first 90 minutes, the signal intensity kept increasing upon the addition of 200, 400 and 800 U mL^{-1}

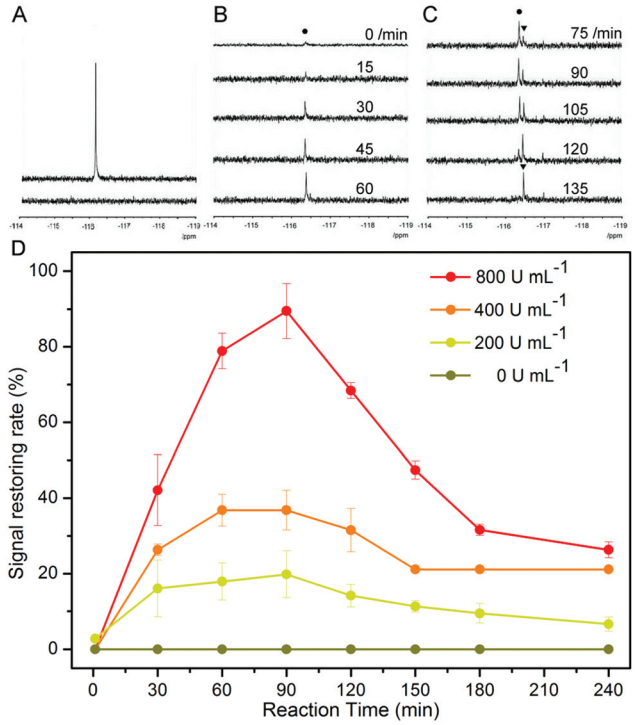


Fig. 2 ^{19}F-NMR signal (A) could be turned off by adding phosphatase to the solution of **1** and then (B, C) turned on by adding tyrosinase. (D) Time-course of the signal restoring rate in the presence of various concentrations of tyrosinase. (The rates were calculated by comparing the integral of peak area at −116.43 ppm and −116.53 to the peak area of the internal standard of TFA at −75.6 ppm.)

tyrosinase and finally reached the top of about 89.5%, 36.8% and 19.8% signal restoration, respectively. Obviously, the increasing speed was proportional to the concentration of tyrosinase and no signal could be detected when no tyrosinase was added. However, the F-NMR signal began to decrease after 90 minutes and has not resumed any more. We hypothesize that this phenomenon could be explained by two combined factors. On the one hand, tyrosinase gradually became irreversibly inactivated under aerobic conditions, induced by its physiological substrate L-dopa.[15] After the addition, tyrosinase of higher concentration would immediately convert a larger amount of compound **2** into polyphenol, which was more hydrophilic and resulting in the stronger F-NMR signal. Then this L-dopa analogue would lead to the inactivation of tyrosinase, stopping the supply of the signal compound. On the other hand, polyphenol spontaneously oxidized into quinone, which reduced the signal intensity due to its poorer solubility in water. These two factors together caused the result that F-NMR signal restoration of our nanospheres first increased and then decreased. This observation indicated that using our nanospheres described in this study to detect tyrosinase should be operated within 90 minutes.

In order to test the biocompatibility of the nanospheres and the resulting products by tyrosinase, we performed an MTT assay of NIH·3T3 mouse fibroblast cells treated with

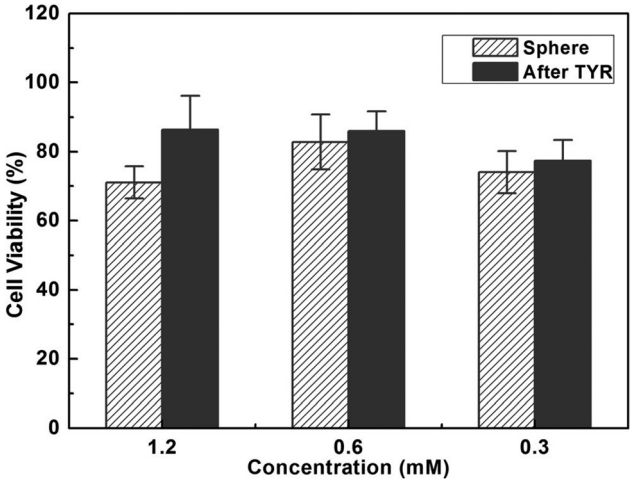


Fig. 3 Cell viability of NIH 3T3 mouse fibroblast cells treated with different concentrations of nano-spheres before and after treating with tyrosinase for 24 hours (determined by the MTT assay).

different concentrations of the compounds to evaluate their cytotoxicity. The results in Fig. 3 indicated that about 80% of 3T3 cells were alive after being cultured in Dulbecco's modified Eagle's medium (DMEM) containing compounds at concentrations lower than 1.2 mM for 24 hours. These observations indicated the low toxicity of compounds to 3T3 cells at these concentrations.

In summary, we have developed self-assembled ^{19}F-containing nanospheres that can be applied for detecting the enzyme tyrosinase through ^{19}F-NMR signal turn on. There was no fluoride in the human body; thus F-NMR turn on probes will have great potential for imaging applications. Many peptides with phenylalanine (F) can self-assemble into nanostructures. Due to the chemical similarity of F and fF, peptides with fF may also self-assemble into well-defined nanostructures with silence in F-NMR signal. Using enzymes or proteins to digest or bind to self-assembled peptides can lead to the disassembly of these nanostructures, leading to F-NMR signal turn on. We therefore imagine that responsive self-assembling systems containing fluoride atoms would show great potential for detection of many important analysts.

This work was supported by the National Natural Science Foundation of China (51222303 and 51373079) and Funds for National Basic Science Personnel Training (J1103503).

Notes and references

1 A. G. Cheetham, P. Zhang, Y.-a. Lin, L. L. Lock and H. Cui, *J. Am. Chem. Soc.*, 2013, **135**, 2907–2910; A. Altunbas, S. J. Lee, S. A. Rajasekaran, J. P. Schneider and D. J. Pochan, *Biomaterials*, 2011, **32**, 5906–5914.
2 X. Li, J. Li, Y. Gao, Y. Kuang, J. Shi and B. Xu, *J. Am. Chem. Soc.*, 2010, **132**, 17707–17709.
3 A. Ghosh, M. Haverick, K. Stump, X. Yang, M. F. Tweedle and J. E. Goldberger, *J. Am. Chem. Soc.*, 2012, **134**, 3647–3650; Y. Gao, J. Shi, D. Yuan and B. Xu, *Nat. Commun.*,

2012, **3**, 1033; Y. Takaoka, K. Kiminami, K. Mizusawa, K. Matsuo, M. Narazaki, T. Matsuda and I. Hamachi, *J. Am. Chem. Soc.*, 2011, **133**, 11725–11731; Y. Takaoka, Y. Sun, S. Tsukiji and I. Hamachi, *Chem. Sci.*, 2011, **2**, 511–520; K. Mizusawa, Y. Ishida, Y. Takaoka, M. Miyagawa, S. Tsukiji and I. Hamachi, *J. Am. Chem. Soc.*, 2010, **132**, 7291–7293.

4 C. Yan and D. J. Pochan, *Chem. Soc. Rev.*, 2010, **39**, 3528–3540; T. D. Sargeant, M. O. Guler, S. M. Oppenheimer, A. Mata, R. L. Satcher, D. C. Dunand and S. I. Stupp, *Biomaterials*, 2008, **29**, 161–171; L. Haines-Butterick, K. Rajagopal, M. Branco, D. Salick, R. Rughani, M. Pilarz, M. S. Lamm, D. J. Pochan and J. P. Schneider, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 7791–7796; S. Zhang, *Nat. Biotechnol.*, 2003, **21**, 1171–1178.

5 K. Rajagopal, M. S. Lamm, L. A. Haines-Butterick, D. J. Pochan and J. P. Schneider, *Biomacromolecules*, 2009, **10**, 2619–2625.

6 M. S. Lamm, N. Sharma, K. Rajagopal, F. L. Beyer, J. P. Schneider and D. J. Pochan, *Adv. Mater.*, 2008, **20**, 447–451; Y. Chen, B. Li, X. Wu, X. Zhu, M. Suzuki, K. Hanabusa and Y. Yang, *Chem. Commun.*, 2008, 4948–4950; Y. Yang, M. Nakazawa, M. Suzuki, H. Shirai and K. Hanabusa, *J. Mater. Chem.*, 2007, **17**, 2936–2943; Y. Yang, M. Suzuki, H. Shirai, A. Kurose and K. Hanabusa, *Chem. Commun.*, 2005, 2032–2034; M. Reches and E. Gazit, *Science*, 2003, **300**, 625–627.

7 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; W. Cao, X. Zhang, X. Miao, Z. Yang and H. Xu, *Angew. Chem., Int. Ed.*, 2013, **125**, 6353–6357; J. Kopeček and J. Yang, *Angew. Chem., Int. Ed.*, 2012, **51**, 7396–7417; J. Wu, Y. Zou, C. Li, W. Sicking, I. Piantanida, T. Yi and C. Schmuck, *J. Am. Chem. Soc.*, 2012, **134**, 1958–1961; J. Wu, A. Zawistowski, M. Ehrmann, T. Yi and C. Schmuck, *J. Am. Chem. Soc.*, 2011, **133**, 9720–9723; D. W. Löwik, E. Leunissen, M. Van den Heuvel, M. Hansen and J. C. van Hest, *Chem. Soc. Rev.*, 2010, **39**, 3394–3412; X. Li, Y. Gao, Y. Kuang and B. Xu, *Chem. Commun.*, 2010, **46**, 5364–5366; 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. Ulijn, *Nat. Chem.*, 2010, **2**, 1089–1094; J. H. Collier, J. S. Rudra, J. Z. Gasiorowski and J. P. Jung, *Chem. Soc. Rev.*, 2010, **39**, 3413–3424; E. F. Banwell, E. S. Abelardo, D. J. Adams, M. A. Birchall, A. Corrigan, A. M. Donald, M. Kirkland, L. C. Serpell, M. F. Butler and D. N. Woolfson, *Nat. Mater.*, 2009, **8**, 596–600; R. J. Williams, A. M. Smith, R. Collins, N. Hodson, A. K. Das and R. V. Ulijn, *Nat. Nanotechnol.*, 2008, **4**, 19–24; A. R. Hirst, B. Escuder, J. F. Miravet and D. K. Smith, *Angew. Chem., Int. Ed.*, 2008, **47**, 8002–8018.

8 J. B. Matson and S. I. Stupp, *Chem. Commun.*, 2011, **47**, 7962–7964.

9 S. C. Bremmer, J. Chen, A. J. McNeil and M. B. Soellner, *Chem. Commun.*, 2012, **48**, 5482–5484; J. Chen, W. Wu and A. J. McNeil, *Chem. Commun.*, 2012, **48**, 7310–7312; X.-D. Xu, B.-B. Lin, J. Feng, Y. Wang, S.-X. Cheng, X.-Z. Zhang and R.-X. Zhuo, *Macromol. Rapid Commun.*, 2012, **33**, 426–431.

10 J. Gao, W. Zheng, D. Kong and Z. Yang, *Soft Matter*, 2011, **7**, 10443–10448.

11 Y. Takaoka, T. Sakamoto, S. Tsukiji, M. Narazaki, T. Matsuda, H. Tochio, M. Shirakawa and I. Hamachi, *Nat. Chem.*, 2009, **1**, 557–561.

12 H. Wang, Z. Wang, D. Song, J. Wang, J. Gao, L. Wang, D. Kong and Z. Yang, *Nanotechnology*, 2010, **21**, 155602.

13 H. M. Wang, C. H. Yang, M. Tan, L. Wang, D. L. Kong and Z. M. Yang, *Soft Matter*, 2011, **7**, 3897–3905.

14 J. Turkevich, P. C. Stevenson and J. Hillier, *Discuss. Faraday Soc.*, 1951, **11**, 55–75.

15 Y. Tomita, A. Hariu, C. Mizuno and M. Seiji, *J. Invest. Dermatol.*, 1980, **75**, 379–382; J. L. Muñoz-Muñoz, J. R. Acosta-Motos, F. Garcia-Molina, R. Varon, P. A. Garcia-Ruíz, J. Tudela, F. Garcia-Cánovas and J. N. Rodríguez-López, *Biochim. Biophys. Acta, Proteins Proteomics*, 2010, **1804**, 1467–1475.

