Chinese Chemical Letters 32 (2021) 1025–1028

Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

Communication

Ultra-stable and multistimuli-responsive nanoparticles coated with zwitterionic pillar[n]arene for enhanced cellular uptake

Yupeng Jiao, Shang Lan, Da Ma*

Department of Chemistry, Fudan University, Shanghai 200433, China

ARTICLE INFO

Article history:
Received 19 June 2020
Received in revised form 13 July 2020
Accepted 4 August 2020
Available online 6 August 2020

Keywords:
Host-guest systems
Stimuli responsiveness
Zwitterionic ligands
Gold nanoparticles
Cellular uptake

ABSTRACT

Nanoparticle surface property is crucial for circulation stability, cellullar uptake and other biological characteristics. Zwitterionic pillar[n]arenes (**ZP*n*s**) were used to coat gold nanoparticles (GNPs) *via* host-guest interaction. The resulting GNPs demonstrated higher stability in blood serum compared to polyethylene glycol (PEG)-coated GNPs. **ZP*n***-coated GNPs were responsive to UV-irradiation, competitive displacement and acidic pH. UV-irradiation or competitive displacement could lead to the removal of **ZP*n*** coating to expose GNPs, which enhanced cell uptake efficiency by 5.9- and 7.4-fold, respectively.

Nanoparticles are of great importance for biomedical applications, including drug delivery and bioimaging [1,2]. Surface property of nanoparticles is considered to be the deciding factor for biophysicochemical interactions at the nano-bio interface [3]. Manipulation of nanoparticle surface characteristics may influence circulation stability and cell uptake efficiency [4–6]. To improve stability and avoid nonspecific adsorption of serum proteins or other biomolecules during circulation, nanoparticles are often coated with polyethylene glycol (PEG) or other "nonfouling" biomaterials to achieve "stealth" [7]. When reaching tumor or other target tissues, it is desired to enhance cell uptake and tissue accumulation [8]. Stimuli-responsive "sheddable" shell of PEG or other nonfouling coating material has been used to achieve the goal of cell uptake enhancement.

Although widely used, PEG suffers from limitations, such as susceptibility to oxidation damage and anti-PEG antibodies [9,10]. Zwitterionic polymeric materials, such as poly(carboxybetaine), are second generation nonfouling biomaterials, which demonstrate ultralow-fouling toward serum proteins [11–13]. To render PEG- or zwitterionic polymer-coated nanoparticles stimuli-responsive, relatively complex design is required. For example, nonfouling and "charge-conversion" polymers have been used to achieve stimuli-responsive enhancement of cell uptake [14–16]. It is highly desired to develop a simple strategy to simultaneously ensure high stability in blood serum, and stimuli-induced cell uptake improvement in target tissues.

Host-guest interaction is a non-covalent force to conveniently assemble complex nanostructures with multistimuli-responsiveness [17–26]. We decided to explore the application of pillar[n]arene (PA[n])-based zwitterionic host molecules as nonfouling coating materials [27–35]. Gold nanoparticles (GNPs) were used as model nanoparticles, and nanoparticle surface was modified with azobenzene-based guest. Zwitterionic PA[*n*] (**ZP*n***) was used to coat GNPs *via* host-guest interaction [36]. The resulting GNPs were discovered to be ultra-stable in blood serum. UV-irradiation, competitive displacement or acidic pH was used to manipulate surface property of GNPs by the removal of **ZP*n***-coating layer or conversion of surface charge. Stimuli-induced conversion of GNP surface characteristics was confirmed to significantly enhance GNP uptake by human cancer cells *in vitro*.

As shown in Fig. 1a, **ZP5** and **ZP6** were synthesized by substitution reaction of bromo-substituted PA[*n*]s, followed by hydrolysis of ester. **ZP*n***s are composed of equal numbers of carboxylic acid and pyridinium groups. Under neutral physiological condition, carboxylic acid groups are deprotonated, and **ZP5** and **ZP6** possess equal amount of cationic and anionic groups. By contrast, at acidic pH, carboxylic acid groups are protonated to render the host molecule cationic. For comparison purpose, carboxylated PA[*n*]s (**WP5-WP6**) were prepared (Fig. 1b). Azobenzene-based neutral guest **1** was synthesized, and conjugated to GNP surface by gold-thiol bond. Azobenzene is known to undergo reversible conversion between *trans*- and *cis*-configurations under UV or visible irradiation [37]. GNPs were prepared by citric

* Corresponding author.
E-mail address: dama@fudan.edu.cn (D. Ma).

https://doi.org/10.1016/j.cclet.2020.08.001

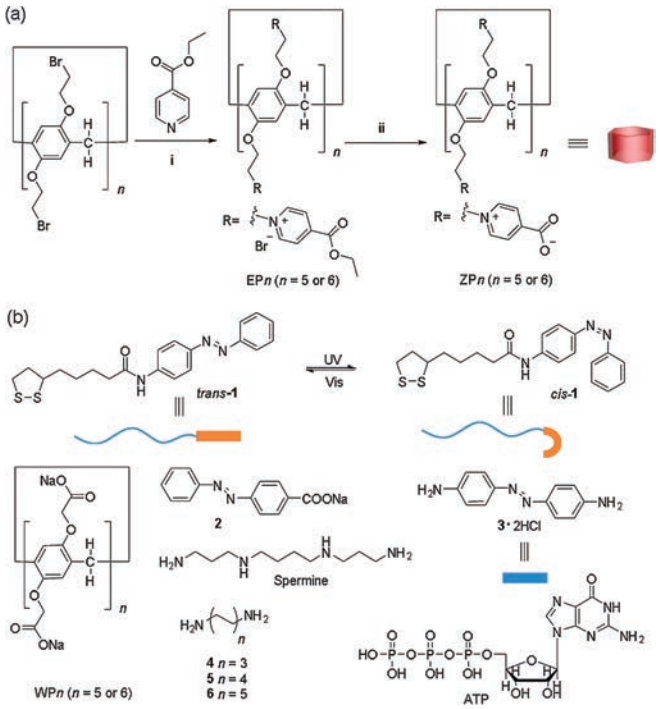


Fig. 1. (a) Synthetic procedure for **ZP5-ZP6**. i) EtOH, 90 °C, 48 h. ii) NaOH, H_2O, reflux, 24 h. (b) Chemical structures of carboxylated PA[*n*]s **WP5, WP6**, guests **1-6**, and biomolecules spermine and ATP.

acid-catalyzed reduction reaction of $HAuCl_4$. Subsequently, guest **1** was conjugated to GNPs. Transmission electron microscopy (TEM) showed that GNPs were relatively uniform with an average size of 16 nm (Fig. S32 in Supporting information). Zeta-potential indicated that guest **1**-conjugated GNPs (bare GNPs) were negatively charged (−29 mV). **ZP5** and **ZP6** were incubated with GNPs to conjugate zwitterionic host molecules by host-guest interaction (Fig. 2a). **ZP6** coating enhanced ζ-potential to −22 mV (Fig. S34 in Supporting information). PEG- and **WP5/WP6**- coated GNPs were prepared for comparison purpose. Similar GNP morphology was observed by TEM (Fig. S32). While **WP6**-coated GNPs were highly negatively charged (−26 mV), PEG-coated GNPs were slightly negatively charged (−7 mV).

First, we investigated host-guest chemistry in sodium phosphate buffer. Neutral guest **1** was poorly soluble in water. We used diamino azobeneze (guest **3**, positively charged) and carboxylated azobenzene (guest **2**, negatively charged) as model guests. **ZP5-ZP6** and **WP5-WP6** were used as host molecules. Neutral pH (7.4) and acidic pH (5.5) were used to study the influence of solvent acidity. Binding constant (K_a) value was determined by indicator displacement assay with rhodamine 6 G or acridine orange as the

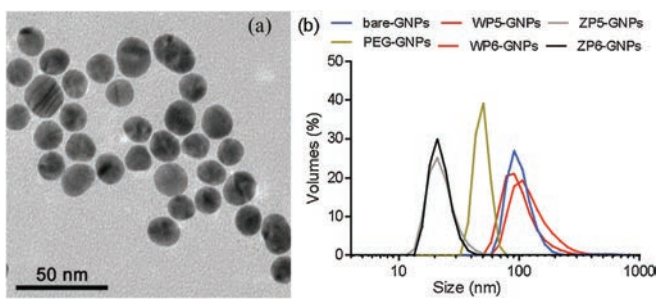


Fig. 2. (a) TEM images of **ZP6**-GNPs. (b) Hydrodynamic diameter (nm) of bare-GNPs, **WP6**-GNPs, **ZP6**-GNPs and PEG-GNPs after incubation in FBS (50%) for 30 h.

Table 1
The value of K_a (L/mol) for the complex of **ZP5**-**ZP6** and guests **2**-**6** or biomolecules spermine/ATP at neutral (pH 7.4) or acidic (pH 5.5) condition.

Guest	ZP6	ZP5
2	$(4.0 \pm 0.9) \times 10^5$	$(1.1 \pm 0.2) \times 10^5$
3	$(1.8 \pm 0.3) \times 10^5$	$(1.5 \pm 0.3) \times 10^5$
4	$(1.0 \pm 0.2) \times 10^4$	$(5.1 \pm 1.0) \times 10^4$
5	$(4.8 \pm 1.1) \times 10^2$	$(8.2 \pm 2.0) \times 10^2$
6	$(4.7 \pm 1.2) \times 10^2$	$(1.2 \pm 0.3) \times 10^3$
Spermine	$(1.8 \pm 0.7) \times 10^2$	$(6.4 \pm 1.0) \times 10^2$
ATP	$(2.5 \pm 1.7) \times 10^2$	$(1.0 \pm 0.6) \times 10^2$
2*	$(2.2 \pm 0.6) \times 10^5$	–
3*	$(2.4 \pm 0.3) \times 10^5$	–

* Determined at pH 5.5. -: not determined.

dye. As shown in Table 1, **ZP5-ZP6** could tightly bind guests **2–3** with similar binding affinity. Upfield shift of azobenzene proton resonances in ^{1}H NMR spectra showed that *trans*-azobenzene were encapsulated by **ZP*n*** cavity (Fig. S31 in Supporting information). By contrast, *cis*-isomer of azobenzene could not be encapsulated by **ZP*n*** based on ^{1}H NMR spectroscopy. It was discovered that acidic pH had limited impact toward K_a value. Therefore, **ZP5-ZP6** were high affinity host molecules for *trans*-isomers of azobenzene guests at neutral or acidic pH. Similarly, **WP5-WP6** could bind guest **3** with high affinity (Figs. S29 and S30 in Supporting information).

Model cationic guests **4–6**, and biomolecules spermine and adenosine triphosphate (ATP) were chosen to further explore host-guest chemistry. **ZP5-ZP6** showed selective binding toward guests **4–6** with different lengths. **ZP5-ZP6** demonstrated significantly lower binding affinity toward positively-charged biomolecule spermine and negatively-charged biomolecule ATP, compared to azobenzene-guests **2–3**. The selective binding may ensure stable supramolecular encapsulation under complex physiological condition.

Second, stability of nanoparticles was evaluated in blood serum. Fetal bovine serum (FBS) was used to mimic blood serum condition. Bare or coated GNPs were incubated in 50% FBS at 37 °C, and aliquots of nanoparticle suspension were extracted to separate GNPs from solvent by centrifugation. Supernatant was removed and GNPs were re-suspended in ultrapure water to determine nanoparticle size by dynamic light scattering (DLS). As shown in Figs. 2b and 3a, after 30 h incubation, hydrodynamic size of bare GNPs significantly increased in FBS. **WP5/WP6**-coated GNPs also had significant enhancement in size. These three groups of GNPs were lack of nonfouling-coating layer to protect them from nonspecific adsorption of serum proteins and other biomolecules in FBS, which resulted in aggregation and nanoparticle size growth. By sharp contrast, **ZP5/ZP6**-coated GNPs had a constant hydrodynamic size of 24 nm for up to 48 h. The "stealth" effect of **ZP5-ZP6** coating was superior compared to that of PEG:PEG-coated GNPs showed an increase in hydrodynamic size after 10 h, while **ZP*n***-coated GNPs maintained their size. Consequently, **ZP*n***s were excellent nonfouling coating material for GNPs.

Host-guest interaction is non-covalent, and could be unstable in blood serum, especially when exposed to competitive biomolecular guests. To determine the stability of surface coating, **ZP6**-coated GNPs were incubated in FBS for 0.5 h, and isolated from the solution by centrifugation. After repeated for three times, GNPs were re-suspended in fresh FBS (50%), and size distribution was monitored by DLS. As shown in Fig. 3b, nanoparticle size maintained at 24 nm for up to 7 d, which demonstrated that **ZP6**-coating layer was retained even after extensive washing by FBS. We attributed the stability to high affinity binding between **ZP*n*** and guest **1**. Although FBS is composed of complex biomolecular species, minimal

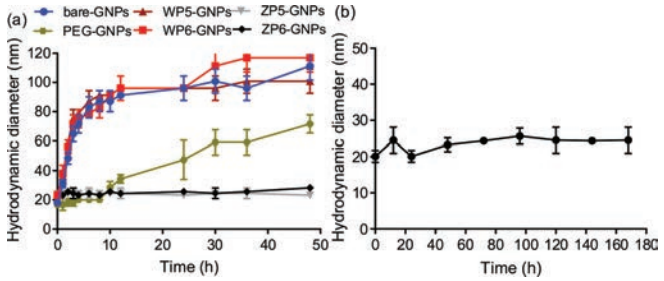


Fig. 3. (a) Z_{avg} hydrodynamic diameter (nm) of bare-GNPs, **WP5**-GNPs, **WP6**-GNPs, **ZP5**-GNPs, **ZP6**-GNPs and PEG-GNPs in FBS (50%). (b) Z_{avg} hydrodynamic diameter (nm) of **ZP6**-GNPs in FBS (50%), after being washed with FBS for three times.

competitive displacement occurred. We expect **ZP*n***-coated GNPs to be stable during blood circulation.

Next, we studied the stimuli-responsiveness of **ZP*n*** nonfouling coating layer. **ZP5/ZP6-**coated GNPs were incubated in 10% FBS, and UV-irradiation (365 nm) was applied for 2 h. Hydrodynamic size of GNPs in water was monitored by DLS. As shown in Fig. 4a, hydrodynamic size of nanoparticles significantly increased. We believe the reason was that guest **1** transformed to *cis*-isomer, and **ZP*n*** coating layer was removed from GNP surface, which led to serum protein adsorption and GNP aggregation. Subsequently, GNP suspension was irradiated with visible light for 2 h to allow *cis*-azobenzene to convert to *trans*-isomer. GNPs regained smaller hydrodynamic size in ultrapure water, which showed that **ZP*n***-nonfouling coating layer attached to nanoparticle surface *via* host-guest interaction. This reversible process could be repeated for up to six cycles.

Similarly, **ZP*n***-coated GNPs were responsive to competitive displacement and acidic pH. As shown in Fig. 4b, guest **3** was used as competitive guest, and added to a suspension of **ZP6**-coated GNPs in 10% FBS. It was discovered that the addition of competitive guest **3** would result in the enhancement in GNP hydrodynamic size. To study responsiveness to acidic pH, **ZP6-** or PEG-coated GNPs were incubated in 10% FBS at pH 7.0 or pH 5.5. As shown in Fig. 4b, at pH 5.5, **ZP6-**coated GNPs increased in hydrodynamic size, while PEG-coated GNPs retained their nonfouling ability. Based on K_a value for the complex of **ZP6** and guests **2–3** under neutral and acidic conditions, we believe **ZP6** formed stable supramolecular complex with guest **1** on GNPs at acidic pH (Table 1). The reason for this pH-responsiveness was the protonation of carboxylic acid groups, and conversion from zwitterionic to cationic host molecule. As shown in Fig. S34, ζ-potential of **ZP6**-coated GNPs increased from −26 mV at pH 7.4 to −16 mV at pH 5.5. Similar observations had been reported for zwitterionic polymer-coated GNPs in literature [14].

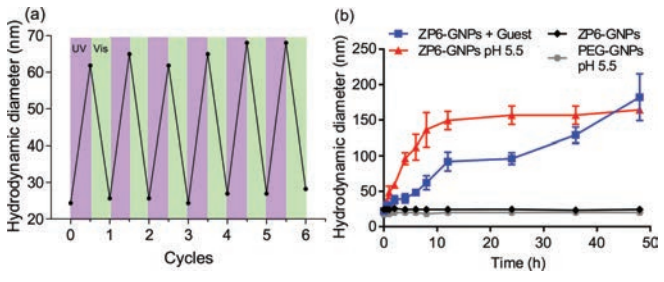
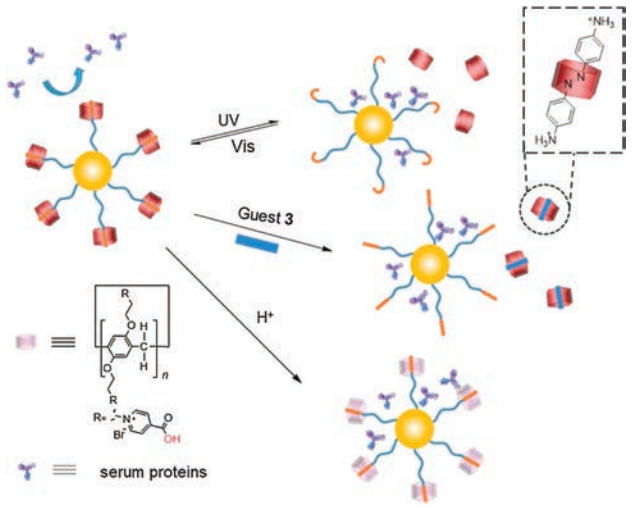


Fig. 4. (a) Z_{avg} hydrodynamic diameter (nm) of **ZP6**-GNPs in FBS (10%) treated with UV/visible irradiation. (b) Z_{avg} hydrodynamic diameter (nm) of **ZP6**-GNPs with or without guest **3**, **ZP6**-GNPs at pH 5.5, PEG-GNPs at pH 5.5; condition: FBS (10%). Temperature: 37 °C. Error bar was calculated based on triplicated measurements.



Scheme 1. Cartoon depicting surface coating of **GNP**s with **ZP6** *via* host-guest interaction to achieve nonfouling effect and multistimuli responsiveness.

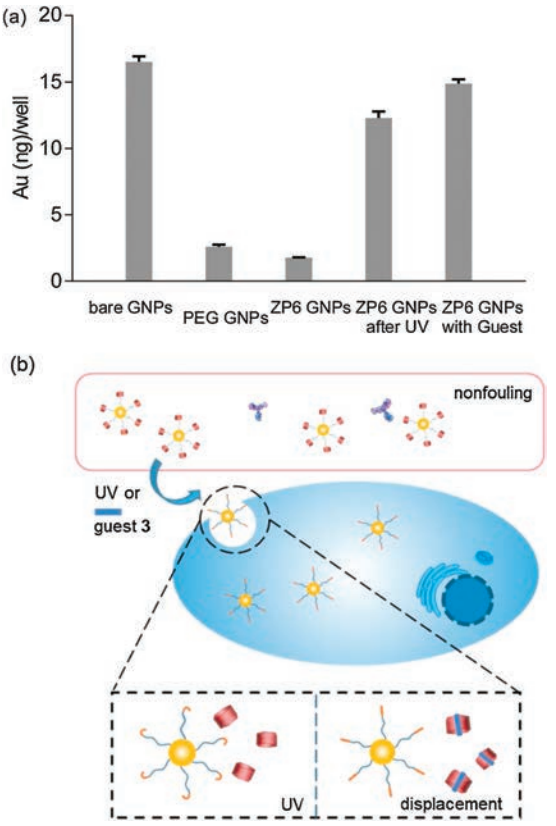


Fig. 5. (a) Cell uptake of bare-GNPs, PEG-GNPs, **ZP6**-GNPs, **ZP6**-GNPs after UV and **ZP6**-GNPs with guest measured by ICP-AES. Each data point represents an average value ± standard deviation from three independent measurements. (b) Cartoon depicting stable **ZP6**-coated GNPs in blood serum, and UV- or guest-induced enhancement in cell uptake.

As summarized in Scheme 1, **ZP*n***-coated GNPs were responsive to three stimuli types: photo-irradiation, competitive guest and acidic pH. Under stimuli, nonfouling and ultra-stable GNPs could expose inner core or undergo surface charge conversion, which may be used to enhance interaction between GNP and biological interface.

Lastly, we investigated the influence of **ZP*n***-based nonfouling coating layer toward cell uptake efficiency of GNPs. Nonfouling coating layer of **ZP*n*** could render nanoparticles "stealth" to protect them from immunological clearance during circulation in blood (Fig. 5b). When reaching target tissues, an enhanced cell uptake of GNPs was preferred to improve therapeutic efficacy or chromophore accumulation. Biocompatibility of **ZP5-ZP6** was evaluated by using human normal cell line MRC-5, and host molecules were confirmed to be nontoxic (Fig. S35 in Supporting information). Human cancer HeLa cell line was used to determine cell uptake efficiency. HeLa cells were incubated with bare or coated GNPs, which were subsequently washed and lysed to determine GNP cell uptake by inductively coupled plasma atomic emission spectroscopy (ICP-AES). As shown in Fig. 5a, bare GNPs had significantly higher cell uptake efficiency compared to that of **ZP6**- or PEG-coated GNPs.

We studied the influence of stimuli-responsiveness toward cell uptake. **ZP6**-coated GNPs were treated with UV-irradiation or competitive guest **3** before incubation with cells, and GNP uptake was determined by ICP-AES. It was discovered that UV-irradiation and competitive displacement resulted in an enhancement of cell uptake by 5.9- and 7.4-fold, respectively. Acid-induced cell uptake enhancement of zwitterionic polymer-coated GNPs was reported in literature [14]. Consequently, external stimuli could lead to the conversion of surface characteristics to improve uptake efficiency by tumor cells. Surface property was reported to be critical for GNP endocytosis pathways and cell uptake efficiency [38]. We believe the removal of **ZP6**-nonfouling coating could expose GNP-core, and enhance interaction with cell membrane receptors or even alter internalization pathway. By using host-guest interaction, nanoparticles were ultra-stable in blood serum due to nonfouling effect of zwitterionic host molecules, and efficiently internalized by tumor cells after applying stimuli.

In conclusion, we report a new multistimuli-responsive nonfouling coating strategy for nanoparticles with zwitterionic PA[*n*]s. By coating with **ZP*n***, nanoparticles were ultra-stable in blood serum. UV-irradiation, competitive displacement or acidic pH could be used to switch off **ZP*n***-nonfouling effect, by which enhancement of cell uptake may be achieved. This strategy may be explored for targeted-delivery of therapeutic or diagnostic agents *in vivo*.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors are grateful to National Natural Science Foundation of China (Nos. 21672042 and 21921003) for financial support.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.cclet.2020.08.001.

References

[1] W. Fan, B. Yung, P. Huang, X. Chen, Chem. Rev. 117 (2017) 13566–13638.
[2] G. Chen, I. Roy, C. Yang, P.N. Prasad, Chem. Rev. 116 (2016) 2826–2885.
[3] A.E. Nel, L. Mädler, D. Velegol, et al., Nat. Mater. 8 (2009) 543–557.
[4] I. Banerjee, R.C. Pangule, R.S. Kane, Adv. Mater. 23 (2011) 690–718.
[5] M.B. Flanagan, M. Lundqvist, J. Stigler, T. Cedervall, T. Bergga, ACS Nano 9 (2011) 7503–7509.
[6] B.A. Hucknall, S. Rangarajan, A. Chilkoti, Adv. Mater. 21 (2009) 2441–2446.
[7] H. Otsuka, Y. Nagasaki, K. Kataoka, Adv. Drug Deliv. Rev. 64 (2012) 246–255.
[8] J. Chen, J. Ding, Y. Wang, et al., Adv. Mater. 29 (2017) 1701170.
[9] E. Ostuni, R.G. Chapman, R.E. Holmlin, S. Takayama, G.M. Whitesides, Langmuir 17 (2001) 5605–5620.
[10] P. Zhang, F. Sun, S. Liu, S. Jiang, J. Control. Release 244 (2016) 184–193.
[11] S. Jiang, Z. Cao, Adv. Mater. 22 (2010) 920–932.
[12] W. Yang, L. Zhang, S. Wang, A.D. White, S. Jiang, Biomaterials 30 (2009) 5617–5621.
[13] L. Zhang, Z. Cao, T. Bai, et al., Nat. Biotechnol. 31 (2013) 553–556.
[14] T. Mizuhara, K. Saha, D.F. Moyano, et al., Angew. Chem. Int. Ed. 54 (2015) 6567–6570.
[15] Y. Yuan, C. Mao, X. Du, et al., Adv. Mater. 24 (2012) 5476–5480.
[16] J.Z. Du, T.M. Sun, W.J. Song, J. Wu, J. Wang, Angew. Chem. Int. Ed. 49 (2010) 3621–3626.
[17] J.M. Zayed, N. Nouvel, U. Rauwald, O.A. Scherman, Chem. Soc. Rev. 39 (2010) 2806–2816.
[18] D.S. Kim, J.L. Sessler, Chem. Soc. Rev. 44 (2015) 532–546.
[19] T. Ogoshi, T. Yamagishi, Y. Nakamoto, Chem. Rev. 116 (2016) 7937–8002.
[20] S.J. Barrow, S. Kasera, M.J. Rowland, J. Del Barrio, O.A. Scherman, Chem. Rev. 115 (2015) 12320–12406.
[21] X. Ma, Y. Zhao, Chem. Rev. 115 (2015) 7794–7839.
[22] G. Yu, K. Jie, F. Huang, Chem. Rev. 115 (2015) 7240–7303.
[23] D. Mao, Y. Liang, Y. Liu, et al., Angew. Chem. Int. Ed. 56 (2017) 12614–12618.
[24] Y. Chen, S. Sun, D. Lu, Y. Shi, Y. Yao, Chin. Chem. Lett. 30 (2019) 37–43.
[25] T. Xiao, L. Zhou, L. Xu, et al., Chin. Chem. Lett. 30 (2019) 271–276.
[26] L.L. Zhao, Y. Han, C.G. Yan, Chin. Chem. Lett. 31 (2019) 81–83.
[27] L. Gao, M. Li, S. Ehrmann, Z. Tu, R. Haag, Angew. Chem. Int. Ed. 58 (2019) 3645–3649.
[28] L. Jiang, X. Huang, D. Chen, et al., Angew. Chem. Int. Ed. 56 (2017) 2655–2659.
[29] Y. Chang, K. Yang, P. Wei, et al., Angew. Chem. Int. Ed. 53 (2014) 13126–13130.
[30] D. Cao, Y. Kou, J. Liang, et al., Angew. Chem. Int. Ed. 48 (2009) 9721–9723.
[31] Y. Cao, X. Hu, Y. Li, et al., J. Am. Chem. Soc. 136 (2014) 10762–70769.
[32] S. Li, H. Zhang, X. Xu, Y. Liu, Nat. Commun. 6 (2015) 7590.
[33] L. Chen, W. Si, L. Zhang, et al., J. Am. Chem. Soc. 135 (2013) 2152–2155.
[34] Z. Li, Y. Zhang, C. Zhang, et al., J. Am. Chem. Soc. 136 (2014) 8577–8589.
[35] L. Luo, G. Nie, D. Tian, et al., Angew. Chem. Int. Ed. 128 (2016) 12905–12908.
[36] H. Li, Y.W. Yang, Chin. Chem. Lett. 24 (2013) 545–552.
[37] D. Xia, G. Yu, J. Li, F. Huang, Chem. Commun. 50 (2014) 3606–3608.
[38] Y. Jiang, S. Huo, T. Mizuhara, et al., ACS Nano 10 (2015) 9986–9993.