ChemComm

Dynamic Article Links ▶

COMMUNICATION

Nucleic Acid-mimicking Coordination Polymer for Label-free Fluorescent Activity Assay of Histone Acetyltransferases

Siyu Chen, Yong Li, Yufang Hu, Yitao Han, Yan Huang, Zhou Nie,* Shouzhuo Yao

A novel and label-free fluorescence assay for histone acetyltransferase (HAT) activity was established via in situ generation of nucleic acid-mimicking CoA-Au(I) coordination polymer (CP). Moreover, the potency of this assay for HATs-
10 **targeted drug discovery was proved by screening HATs inhibitor.**

Protein acetylation is an essential post-translational modification (PTM) mechanism in eukaryotic cellular regulation.[1] Histone acetyltransferases (HATs) are a class of enzymes which catalyze
15 the histone acetylation by transfer of an acetyl group from acetyl-CoA to the specific lysine residues within core histone proteins, which play an essential role in the epigenetic regulation of gene expression by mediating chromatin structure.[2] Dysfunction of histone acetylation and the aberrant activity of HATs are often
20 associated with numerous diseases, such as cancers, metabolic syndromes, and neurological disorders.[3] HATs have been emerged as a novel class of drug target. Hence, the detection method to evaluate the activity of HATs is of significance for the epigenetics-related fundamental biochemical research and
25 pharmaceutical development.

Most of conventional methods for activity screening of HATs are based on acetyl-specific antibodies which directly recognize the acetylated peptide[4], such as the colorimetric assays based on the antibody-mediated assembly of gold nanoparticles[4a] and the
30 fluorescent assay using peptide-tailed quantum-dots and acetyl-specific antibody[4b]. However, these methods suffers from some intrinsic shortcomings of antibodies, such as relative high cost, low stability, and high batch-to-batch variability.[5] In recent years, the alternative HATs assays without requirement of antibodies
35 attract increasing interests.[6] One attractive antibody-free strategy is based on quantification of the by-product (non-peptide product) of acetylation reaction, coenzyme A (CoA).[7] Since CoA is a thiol compound composed by cysteine, pantothenate, and adenosine groups, these methods rely on thiol-reactive colorimetric or
40 fluorogenic probes which are simple, quick, and label-free. Nevertheless, the selectivity of these probes might be problematic for CoA measurement because they are specific to only thiol group rather than intact CoA.

Au(I)-thiol coordination polymer (CP) has attracted
45 considerable attentions because of its unique physical properties and importance in various applications, such as acting as precursor for Au nanocrystals synthesis,[8] chemical sensing,[9] and even therapeutic agents.[10] This Au(I)-thiol CP is formed by the reaction of thiols with Au(III), yielding polymeric one-
50 dimensional supermolecular structure with -Au(I)-thiol- repeated segments which is stabilized by Au(I)-thiol interaction and aurophilic Au(I)-Au(I) interaction.[11] Considering its facile preparation and intriguing chain-like structure combining various functional groups of ligands, Au(I)-thiol CP presents a promising
55 material for bio-analysis. However, the applications of Au(I)-thiol CP in biosensing, especially biological enzyme detection, are greatly unexplored.

Herein, we proposed a novel fluorescent assay for probing HAT activity based on in-situ generation of the nucleic-acid
60 mimicking CP of CoA-Au(I), which exhibits highly specificity to intact CoA. Considering the unique structure of CoA containing both the thiol group (in red) and adenine base (in blue),[12] the potential coordination polymer formed by CoA and Au(III) was proposed as the nano-chain-like polymer with multiple adenine
65 bases as the side groups, which is likely analogue to the structure of poly-A single-strained nucleic acid, for instance ribonucleic acid (RNA). Notably and interestingly, we found that, as a nucleic acid analogue, CoA-Au(I) CP can combine with RNA selective dye SYBR Green II (SGII, one of the most sensitive reagents for
70 staining RNA) and emit strong fluorescence (Scheme 1A). This new phenomenon presents a novel and promising mechanism for HATs activity assay by monitoring CoA generation, which might overcome the limitations of conventional methods, for instance
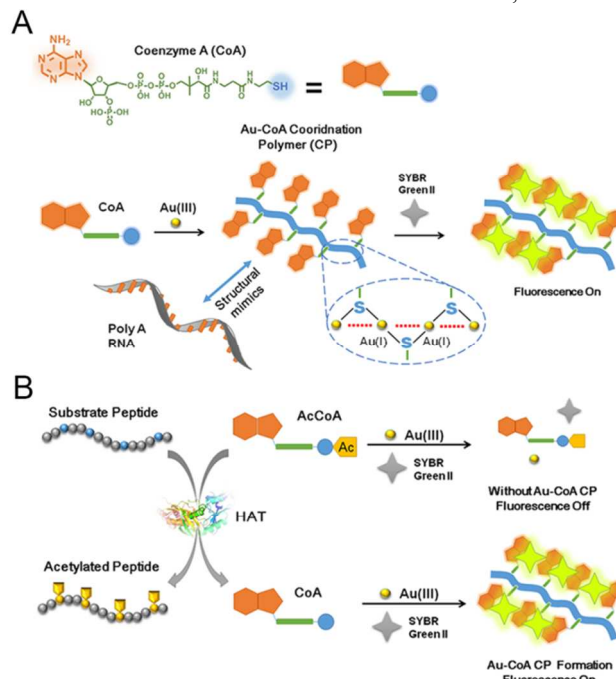
75 **Scheme 1** (A) Formation of RNA-mimicking CoA-Au(I) CP. (B) Illustration of activity analysis of HAT based on CoA-Au(I) CP stained by SGII.
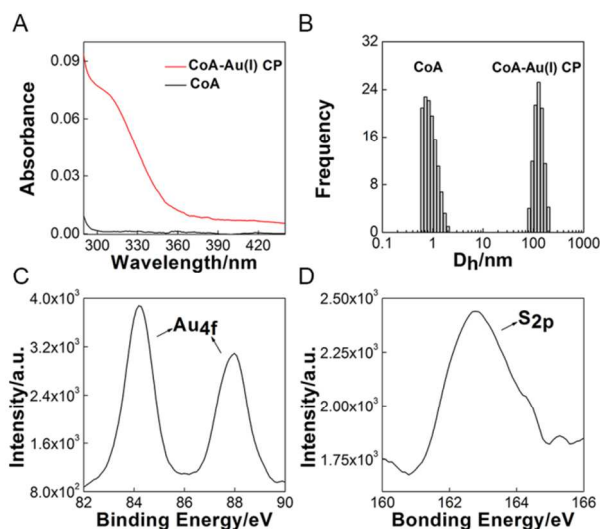
ChemComm Accepted Manuscript

Fig. 1 (A) Absorption spectra of the CoA-Au(I) CP (red line) and CoA (black line). [CoA] = 100 μM, [HAuCl₄] = 50 μM. (B) Size distributions of CoA-Au(I) CP determined by dynamic light scattering (DLS). (C) Au 4f XPS spectrum of CoA-Au(I) CP. (D) S 2p XPS spectrum of CoA-Au(I) CP.
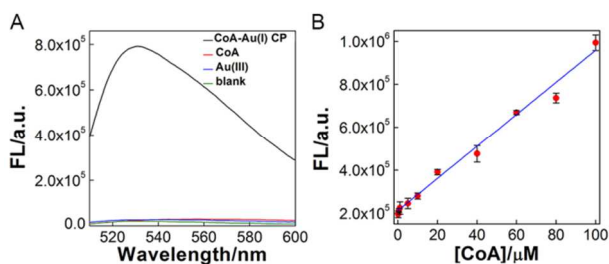

Fig. 2 (A) Fluorescence spectra of the as-prepared CoA-Au(I) CP (λex = 494 nm) in the presence of SGII. [CoA] = 100 μM, [HAuCl₄] = 50 μM. (B) Fluorescence intensity of SGII-stained CoA-Au(I) CP depending on the concentration of CoA. [HAuCl₄] = 50 μM.

the low selectivity of thiol-reactive reagents for CoA detection. The transcriptional activator protein p300 was used as model HAT enzyme in this study. The reactant Ac-CoA contains an acetyl group linked with its sulfhydryl portion, which hampers the generation of CP by blocking the reaction between thiol group and HAuCl₄, thus no fluorescence signal can be measured. In the presence of p300 HAT, the acetyl group was transferred from Ac-CoA to lysine residues on the substrate peptide, producing an acetylated peptide and CoA. CoA was further interacted with HAuCl₄ to form the RNA-mimicking CoA-Au(I) CPs. Then the dye SGII easily combines with CoA-Au(I) CPs and causes the switch-on of fluorescence. The fluorescence enhancement phenomena enable a convenient turn-on detection of p300 activity (Scheme 1B).

To verify the formation of CoA-Au(I) CP, characterization experiments have been done including UV-vis spectroscopy, DLS and XPS experiments. As shown in Fig. 1A, the UV-vis spectra show that the formation of CoA-Au(I) CP causes an obvious shoulder absorption peak at around 320 nm, which could be assigned to the $d_{\sigma*} \rightarrow p_\sigma$ transition.[13] This band is analogous to that observed for diphosphine bridge binuclear Au(I) complexes, which is an indication of aurophilic interaction. DLS data revealed that the reaction of Au(III) and CoA leads to a significant increase of the hydrodynamic radius of species in solution. The average hydrodynamic radius of reaction products is about 127 ± 15 nm, which consists with that of the previously

reported Au(I)-thiol CP, further evidencing that CoA-Au(I) CP was formed (Fig. 1B).[13] XPS was used to investigate the oxidation state of Au atoms in CoA-Au(I) CP (Fig. 1C). Two peaks located at the binding energy of 84.3 eV and 87.9 eV were found, which are consistent with those of $4f_{7/2}$ and $4f_{5/2}$ of Au(I), thereby confirming the successful reduction of Au(III) to Au(I) by CoA. In addition, XPS spectra of CoA-Au CP also evidenced the S 2p binding energy at 163.9 eV, which could be attributed to the formation of -thiol-Au(I)-thiol- linkage (Fig. 1D).[14]

We further explored whether this CP is capable of fluorescence enhancement of SGII, similar to RNA. Interestingly, as we expected, the CoA-Au(I) CP shows strong fluorescence emission intensity at 530 nm in the presence of SGII which is 37.8-fold higher than the SGII-only control. However, the sole CoA or HAuCl₄ results in the negligible fluorescence enhancement, indicating that the formation of CP is required for signal-on of SGII (Fig. 2A). The native polyacrylamide gel electrophoresis (PAGE) with SGII staining further indicated the formation of CP (Fig. S1†). The quantitative comparison of fluorescence intensities reveals that the capability of CoA-Au(I) CP (C_{CoA} = 100 μM, $C_{CoA}:C_{Au}$ = 2:1) to enhance fluorescence of SGII is identical to that of 2 μM 20 nt poly-A RNA (Fig. S2†). These results clearly demonstrated that CoA-Au(I) CP has some RNA-like properties and can be stained by RNA-selective dye SGII, which is not only a potential mechanism for characterizing CoA-based coordination polymer, but highly promising in CoA-related fluorescence detections.

Concentration ratio of CoA and Au(III) (C_{CoA}/C_{Au}), reaction time, temperature, and pH were optimized for efficient preparation of the CP and maximization of fluorescence signal of SGII-stained CP. The effect of HAuCl₄ concentration (0~100 μM) on the fluorescence signal of CoA-Au(I)/SGII was studied with a fixed concentration of CoA (100 μM). The Job plot of fluorescence intensity with increasing concentrate of HAuCl₄ show that the best concentration ratio of CoA and HAuCl₄ for synthesis of CoA-Au(I) CP is 2:1 (Fig. S3†). Meanwhile, the counterpart experiment has also been conducted by changing the CoA concentration with fixed HAuCl₄ concentration (50 μM). The results shown in Fig. 2B suggested the same optimal ratio of CoA/Au (2:1). The extra CoA was thought to be used for reduction of Au(III) to Au(I). Moreover, a clear linearity of CoA concentration ranging from 0.1 μM to 100 μM can be seen in Fig. 2B. The finding of quantitative relationship between fluorescence intensity and CoA concentration provided us a fluorescence analysis platform for biochemical detections of CoA. The reaction time of CoA-Au(I) CP was monitored by the color of the reaction solution which changes from yellow (HAuCl₄) to colorless (CoA-Au(I) CP). Photographs in Fig. S4† show that 8 min is enough for complete reaction. In addition, temperature for synthesis of CoA-Au(I) CP was optimized to be 30 °C according to the results in Fig. S5†. Fig. S6† shows the pH-dependent
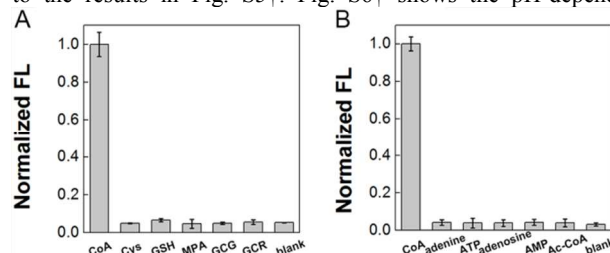

Fig. 3 Normalized fluorescence intensity at 530 nm of the solution of different ligands in reaction with Au(III). (A) Thiol-containing ligands. (B) Adenine-containing ligands. [thiol-containing species] = 100 μM, [adenine-containing species] = 100 μM, [HAuCl₄] = 50 μM, [CoA] = 100 μM.
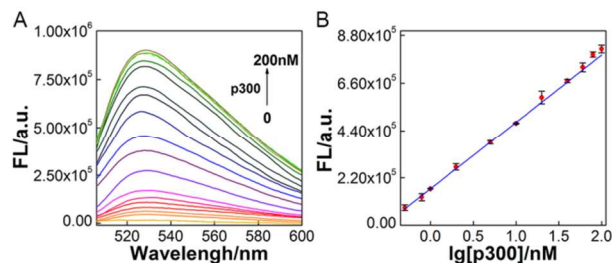
Fig. 4 (A) The fluorescence spectra of the CoA-Au(I) CP-based HAT assay in response to the increasing concentration of p300. (B) Corresponding fluorescence intensity at 530 nm of the HAT assay versus p300 concentrations.
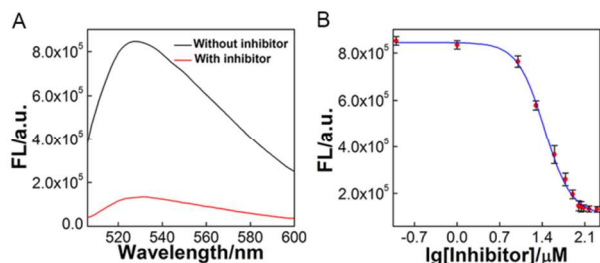

Fig. 5 (A) Fluorescence emission spectra of the HAT assay in response to absence (black line) and presence (red line) of 100 μM anacardic acid (100 nM p300). (B) Changes in fluorescence intensity as a function of p300 inhibitor (anacardic acid) concentration (100 nM p300).

fluorescence response of CoA-Au(I)/SGII. The results indicate that pH 8.0 may be the best pH for detection of CoA-Au(I) CP, while in consideration of reaction pH of most enzymes, pH 7.0 was chosen to be the optimal pH in following experiments.

To examine the necessity of thiol group and adenine moiety in synthesis of unique RNA-mimicking CP, some thiol-containing compounds and adenine-containing compounds were studied for comparison. In this test, each of these compounds reacted with HAuCl$_4$ under the same conditions as CoA did. In the first set of experiments (Fig. 3A), a group of thiol-containing compounds which are also capable of forming coordination polymer with Au ion, including small molecules [cysteine (Cys) and mercaptoacetic (MPA)] and short peptides [glutathione (GSH), Gly-Cys-Gly (GCG), and Gly-Cys-Arg (GCR)], exhibited no obvious fluorescence signal, which is in sharp contrast to CoA. This result proved that SGII staining is highly selective for discriminating CoA from other thiol-containing species because of the special adenine moiety of CoA. As reported, nucleotides can bind to various metal ions via both the hard phosphate ligands and the more soft DNA bases.[15] In order to test whether the adenine nucleotide residue solely is sufficient for SGII staining, the second set of experiments were conducted by replacing CoA with other adenine-containing compounds, such as monophosphate (AMP), adenosine triphosphate (ATP), Ac-CoA, adenine, and adenosine as synthesis ligands. Similar result was obtained (Fig. 3B), and only CoA-Au(I)/SGII exhibit strong fluorescence emission, demonstrating that the interaction between adenine group and Au ion could not switch on the fluorescence of SGII. Furthermore, this result revealed that the Au(I)-thiol interaction to connect CoA monomer to form proposed chain-like structures is of great importance to the fluorogenic property of SGII-bound CoA-derivate CP. Consequently, the formation of RNA-mimicking CP is greatly specific for CoA because it requires two indispensable groups, thiol and adenine, which presents a novel mechanism for selectively fluorescent detection of CoA.

This RNA-mimicking CP was further employed to probe

HATs activity. Under the pre-optimized conditions (500 μM acetyl-CoA, 100 μM peptide, 250 μM HAuCl$_4$), the activity of p300 HAT was quantitatively measured by monitoring CoA generation by acetylation. The relationship between the fluorescence intensity and the concentration of p300 is illustrated in Fig. 4A, and the corresponding calibration curve for p300 activity analysis is shown in Fig. 4B. The proposed fluorescence measurement based on CoA-Au CP and SGII exhibits a high signal-to-background ratio of 19.2 (upon 100 nM p300), which is much higher than that of previously reported HATs assay dependent on thiol-sensitive fluorogenic probes.[7b] Meanwhile, the control experiments were carried out by removing one of the reactants in this HAT reaction mixtures as well (Fig. S7†). The fluorescence spectra show that the absence of any of these reactants results in no fluorescence emission, which provides further evidence for the feasibility of our strategy. The emission intensity gradually increased with the increasing concentration of p300, and there is a linear relationship over the range of 0.5~100 nM (Fig. 4B). The limit of detection (LOD) for p300 is 0.2 nM (signal to noise ratio of 3), indicating that our method is more sensitive than most of HATs assays reported to date (detailed comparison is shown in Table S1†).

In order to simplify this method, the fluorescence signals measured by three different detection approaches were compared: (i) three-step approach: acetyl-CoA and substrate peptide were first treated by p300 for acetylation, then HAuCl$_4$ was introduced to synthesize CoA-Au(I) CP, followed by the addition of SGII for fluorescence signal-out; (ii) two-step approach: acetyl-CoA, substrate peptide, p300 and HAuCl$_4$ were mixed together for enzymatic acetylation and in-situ generation of CoA-Au(I) CP, then SGII was sequentially added after the reaction; (iii) one-pot approach: all the reactants involved in this assay were mixed together for one step measurement (Fig. S8†). The results demonstrated that the fluorescence signals resulting from these three different detection approaches were almost the same, indicating that our assay can be further simplified for facile and rapid detection of HATs activity via direct one-step approach.

Competitive experiments were carried out to examine the potential interference of some typical adenine-containing (AMP, ATP, adenine, adenosine) or thiol-containing compounds (GSH, Cys, MPA) on our assay, since these compounds are ubiquitous in enzymatic reaction buffer or biological fluids. As shown in Fig S9† and S10†, almost no change in the emission intensity was observed when these competitors added, indicating the negligible impact of coexisting adenine-containing or thiol-containing compounds on the signal response of our assay. The selectivity of this HAT assay was also evaluated by challenging the assay with several non-specific enzymes (Fig. S11†). The data showed that none of these enzymes gave any noticeable fluorescence emission, which clearly suggests that these enzymes did not interfere with the detection of p300 and the assay is highly specific for HAT.

Given the importance of HATs in several diseases, such as cancers, Alzheimer's disease (AD), diabetes, and hyperlipidaemia, the sensitive assay for characterization and screening of the HATs inhibitor is crucial for therapeutic investigation. Accordingly, the inhibition effect of p300 inhibitor was determined by the proposed fluorescence assay. In this experiment, a small-molecule inhibitor named anacardic acid was selected as an example. Fluorescence intensity of CoA-Au(I)/SGII decreases about 94.8% with addition of 80 μM anacardic acid (Fig. 5), and an inhibitor concentration-dependent decrease in fluorescence intensity was observed. The IC$_{50}$ value (the concentration of the inhibitor at the half maximal inhibition of the enzyme activity) of anacardic acid toward p300 was

estimated to be 43.3 ± 7.9 μM, which was comparable with the results reported in the previous literature.[6] The result clearly shows that this new assay based on the CoA-Au(I) CP could be applicable for the p300 inhibitor screening.

5   In summary, a novel fluorescence assay for detection of histone acetyltransferase activity has been proposed by facile synthesis of RNA-mimicking CoA-Au(I) CP. We demonstrated for the first time that CoA can serve as a special synthesis ligand to prepare biologically functionalized CP. Because of the unique

10 structure of CoA containing both the thiol and adenine groups, the CoA-Au(I) CP with multiple adenine moieties bears some structural and functional similarity to RNA. We discovered that the as-synthesized CoA-Au(I) CP can interact with RNA-specific dye SGII and emit strong fluorescence, which is highly specific

15 for CoA recognition. The proposed CoA-Au(I) CP was exploited to develop a simple, rapid, and one-step assay for homogeneous detection of HATs activity and screening of HATs inhibitor. This HATs assay presents multifaceted advantages over conventional methods: (i) our assay is antibody-free, label-free, and cost-

20 efficient; (ii) all the assay reagents are commercially available, thus avoiding the sophisticated probe preparation and modification; (iii) our assay is much more specific for CoA recognition in comparison with thiol-reactive probes-based assays. We expect that our strategy could be a promising sensing

25 platform for HAT-related epigenetic research and HAT-targeted drug screening.

Notes and references

35 *State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha, 410082, P. R. China.. Fax: +86-731-88821848; Tel: +86-731-88821626; E-mail: niezhou.hnu@gmail.com*

† Electronic Supplementary Information (ESI) available: [details of any
40 supplementary information available should be included here]. See DOI: 10.1039/b000000x/

1   H. Yuan, R. Marmorstein, *Biopolymers* 2012, **99**, 98-111.

2   B. Li, M. Carey, J. L.Workman, *Cell* 2007, **128**, 707-719.

3   (a) F. J. Dekker, H. J. Haisma, *Drug Discovery Today* 2009, **14**, 942-
45   948; (b) R. B. Selvi, T. K. Kundu, *Biotechnol. J.* 2009, **4**, 375-390.

4   (a) Z. Zhen, L. Tang, H. Long, J. Jiang, *Anal. Chem.* 2012, **84**, 3614-3620. (b) J. E. Ghadiali, S. B Lowe, M. M. Stevens, *Angew. Chem. Int. Ed.* 2011, **50**, 3417-3420.

5   (a) D. Kuninger, J. Lundblad, A. Semirale, P. J. Rotwein, *Biotech.*
50   2007, **131**, 253-260; (b) S. Ali-Si-Ali, S. Ramirez, P. Robin, D. Trouche, A. Harel-Bellan, *Nucleic Acids Res.* 1998, **26**, 3869-3870; (c) Z. Zhen, L. Tang, H. Long, J. H. Jiang, *Anal. Chem.* 2012, **84**, 3614-3620; (d) J. Wu, Y. G. Zheng, *Anal. Biochem.* 2008, **380**, 106-110.

55 6   Y. T. Han, P. Li, Y. T. Xu, H. Li, Z. L. Song, Z. Nie, Z. Chen, S. Z. Yao, DOI: 10.1002/smll.201401989.

7   (a) Y. Kim, K. G. Tanner, J. M. Denu, *Anal. Biochem.* 2000, **280**, 308-314; (b) T. L. Gao, C. Yang, Y. J. G. Zheng, *Anal. Bioanal. Chem.* 2013, **405**, 1361-1371; (c) P. R. Thompson, D. Wang, L. Wang, M.

60   Fulco, N. Pediconi, D. Zhang, W. An, Q. Ge, R. G. Roeder, J. Wong, M. Levrero, V. Sartorelli, T. J. Cotter, P. A. Cole, *Nat. Struct. Mol. Biol.* 2004, **11**, 308-315; (d) R. C. Trievl, F. Y. Li, R. Marmorstein, *Anal. Biochem.* 2000, **287**, 319-328.

8   Y. C. Negishi, K. Nobusada, T. Tsukuda, *J. Am. Chem. Soc.* 2005,
65   **127**, 5261-5270.

9   (a) W. J. Chen, L. Y. Zheng, M. L. Wang, Y. W. Chi, G. N. Chen, *Anal. Chem.* 2013, **85**, 9655-9663; (b) D. L. Liao, J. Chen, H. P. Zhou, Y. Wang, Y. X. Li, C. Yu, *Anal. Chem.* 2013, **85**, 2667-2672; (c) D. H. Li, J. S. Shen, N. Chen, Y. B. Ruan, Y. B. Jiang, *Chem.*
70   *Commun.* 2011, **47**, 5900-5902; (d) J. S. Shen, D. H. Li, Q. G. Cai, Y. B. Jiang, *J. Mater. Chem.* 2009, **19**, 6219-6224.

10   E. E. Langdon-Jones and S. J. A. Pope, *Chem. Commun.* 2014, **50**, 10343-10354.

11   (a) M. Z. Zhu, E. Lanni, N. Garg, M. E. Bier, R. C. Jin, *J. Am. Chem.*
75   *Soc.* 2008, **130**, 1138-1139; (b) K. M. Anderson, A. E. Goeta, J. W. Steed, *Inorg. Chem.* 2007, **46**, 6444-6451.

12   J. Baddiley, E. M. Thain, G. D. Novelli, F. Lipmann, *Nature* 1953, **171**, 76.

13   R. P. Brinas, M. H. Hu, L. P. Qian, E. S. Lymar, J. F. Hainfeld, *J. Am.*
80   *Chem. Soc.* 2008, **130**, 975-982.

14   Z. T. Lou, X. Yuan, Y. Yu, Q. B. Zhang, D. T. Leong, J. Y. Lee, J. P. Xie, *J. Am. Chem. Soc.* 2012, **134**, 16662-16670.

15   A. Lopez and J. W. Liu *J. Phys. Chem. C* 2013, **117**, 3653-3661.