Electronic Supplementary Information (ESI)

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

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EXPERIMENTAL SECTION

1. Materials

Recombinant GST-purified p300 HAT domain, coenzyme A (CoA), acetyl coenzyme A (Ac-CoA), mercaptoacetic acid (MPA), hydrogen tetrachloroaurate trihydrate ($HAuCl_4 \cdot 3H_2O$), carboxypeptidase Y (CPY), horseradish peroxidase (HRP), thrombin, and anacardic acid were purchased from Sigma-Aldrich (Shanghai, China). Chymotrypsin (CHY), adenosine triphosphate (ATP), adenosine monophosphate (AMP), adenosine, adenine, cysteine (Cys), glutathione (GSH), acrylamide, acrylamide/bisacrylamide (39:1, powder), SYBR Green II (SGII) were purchased from Sangon (Shanghai, China). 6× DNA loading buffer was purchased from Tiangen Biotech (Beijing, China). Substrate peptide (RGKGGKGLKGGAKA) and two control peptides (GCG and RCG) were obtained from GL Biochem (Shanghai, China). Poly A RNA (20 nt) was synthesized by Takara (Dalian, China). Other chemicals were all of analytical grade and used without further purification. Ultrapure water (Milli-Q) with a resistivity of 18.2 MΩ from the Millipore Milli-Q system was used as the general solvent throughout the study.

2. Apparatus

Fluorescence measurements were made using 1 cm path length quartz cuvettes on a

Quanta Master™ 4 fluorescence spectrometer (PTI). Absorption spectra were obtained with a UV-vis spectrophotometer (DU 800, Beckman). X-ray photoelectron spectroscopy (XPS) spectra were performed on Thermo Fisher K-Alpha 1063. The hydrodynamic diameter was recorded by dynamic light scattering (DLS) (Malvern). Polyacrylamide gel electrophoresis (PAGE) results were scanned by a ChemiDoc™ MP System (Bio-Rad).

3. Preparation of CoA-Au(I) Coordination Polymer (CP)

The synthesis of CoA-Au(I) CP was carried out in a 100 μL mixture containing freshly prepared aqueous solution of $HAuCl_4 \cdot 3H_2O$ (50 μM), CoA (100 μM), and phosphate buffer (10 mM, pH 7.0). For full reaction of reactants, the mixture was incubated at 30 ^oC with gentle stirring (500 rpm) on a constant temperature magnetic stirrer for 8 min until the light yellow solution turns to clear. After reaction, SGII (1:100, 2 μL) was added to the mixture and incubated for 20 min in dark environment. Then the fluorescence measurement was conducted with excitation at 494 nm. Except for the specific cases mentioned in the text, the fluorescence intensity of all the spectra was measured at 530 nm which is the maximum emission peak of CoA-Au(I) CP/SGII.

4. Characterization of CoA-Au(I) CP

The CoA-Au(I) CP was characterized by X-ray photoelectron spectroscopy and dynamic light scattering (DLS). For the XPS experiment, 50 μL of 100 μM CoA-Au(I) CP was drop to the surface of a slide, and then put it into a clean chemical hood until the aqueous drop dried out. Then the XPS was carried out. For the DLS experiment, 100 μL of 100 μM CoA-Au(I) CP was prepared and recorded. The synthesis conditions were the same as aforementioned.

5. Gel electrophoresis experiment

CoA-Au(I) CP synthesis process was conducted as the same as aforementioned. After CoA-Au(I) CP formation, the sample was diluted with 1 × DNA loading buffer and loaded to a 4% polyacrylamide (PAGE) gel. The electrophoresis was carried in 1×Tris-acetic acid-EDTA (TAE) buffer containing 5 mM Mg^{2+} at a constant voltage mode (85 V) for 1 h. The

gel was stained by SGII for 20 min and scanned by a ChemiDoc™ MP System (Bio-Rad). The control experiment was conducted as the above procedure but with the sole CoA or sole Au(III).

6. The study of reaction concentration ratio of CoA and Au(III)

The reaction concentration ratio of CoA and Au(III) was first studied by fixing CoA concentration at 100 μM, and the fluorescence signal of CoA-Au(I)/SGII was recorded by changing the Au(III) concentration (0, 20, 30, 40, 50, 70, and 100 μM). Counterpart experiment has also been conducted with fixed $HAuCl_4$ concentration at 50 μM, the fluorescence signal of CoA-Au(I)/SGII was recorded by changing the CoA concentration (0, 1, 5, 10, 15, 20, 40, 60, 80, 100, 110, and 120 μM). The CoA-Au(I) CP synthesis conditions were the same as aforementioned except using different concentrations of reactants.

7. Detection of histone acetyltransferases (p300) activity

For p300 activity analysis, p300 of different concentrations (0, 0.1, 0.2, 0.5, 1.0, 10, 20, 40, 60, 80, 100, 120 and 200 nM) was incubated with substrate peptide (200 μM), actyl-CoA (500 μM), $HAuCl_4$ (250 μM), and SGII (1:100, 2 μL) in phosphate buffer (10 mM, pH 7.0) with a total volume of 100 μL at 30 °C for 80 min. After reaction, the mixture was taken out for fluorescence measurement with the conditions similar to those abovementioned. Each experiment was repeated at least three times.

8. Competitive experiments of thiol- and adenine-containing compounds in p300 HAT detection

The study of interference of thiol- and adenine-containing compounds in p300 HAT detection was performed by adding extra thiol-containing compounds (cysteine (Cys), mercaptoacetic acid (MPA), and glutathione (GSH)) or adenine-containing compounds (adenosine triphosphate (ATP), adenosine monophosphate (AMP), adenosine, and adenine). The experimental conditions were the same as those for p300 HAT activity detection except adding extra thiol- or adenine-containing compound to the p300 reaction mixture. The concentration of p300 HAT was 100 nM, the concentration of each interference compound

was 100 μM.

9. Investigation on the selectivity of the proposed HAT assay

The study of selectivity was performed by challenging the proposed assay by protein kinase A (PKA), carboxypeptidase Y (CPY), thrombin (TB), Chymotrypsin (CHY), and horseradish peroxidase (HRP), respectively. The experimental conditions were the same as those for p300 except using different enzyme to replace p300. The concentration of the each enzyme was 10 nM.

10. Detection of histone acetyltransferases (p300) inhibitor

For p300 inhibition analysis, p300 HAT (100 nM), substrate peptide (200 μM), and inhibitor anacardic acid at different final concentrations (0.1, 1, 10, 20, 40, 60, 80, 100, 120, 150, and 200 μM) were pre-incubated for 10 min at room temperature, and then the reaction was initiated by the addition of acetyl-CoA (500 μM), $HAuCl_4$ (250 μM), and SGII (1:100, 2 μL) in phosphate buffer (10 mM, pH 7.0) with a total volume of 100 μL at 30 °C. After incubation of reaction mixture for 80 min, the following processes were then performed as described above.

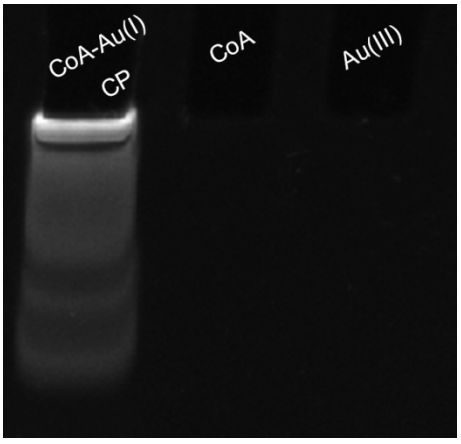

Fig. S1 Native PAGE analysis of the as-synthesized CoA-Au(I) CP. [CoA-Au(I) CP] = 2 mM, [CoA] = 2 mM, [Au(III)] = 1 mM.

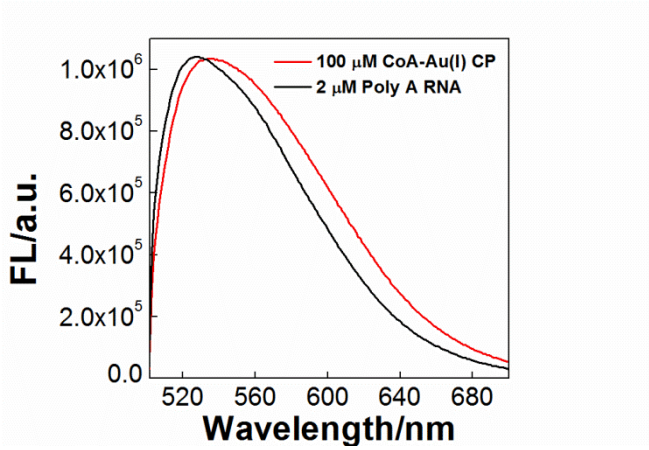

Fig. S2 Fluorescence spectra of 100 μM CoA-Au(I) CP (red line) and 2 μM 20 nt poly-A RNA (black line) in the presence of SGII.

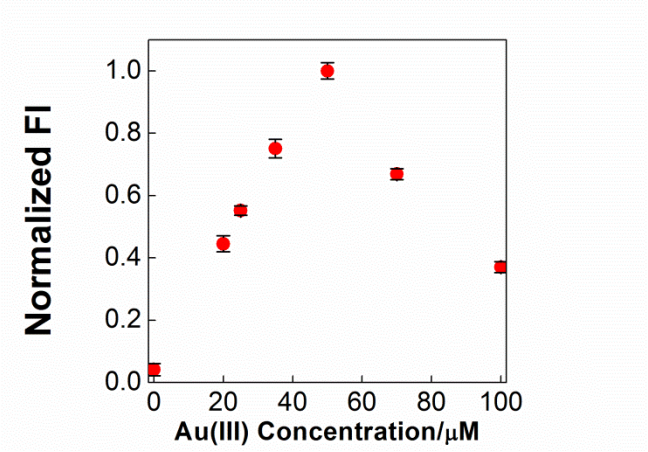

Fig. S3 Normalized fluorescence intensity of SGII-stained CoA-Au(I) CP at 530 nm as a function of different concentration of Au(III). [CoA] = 100 μM

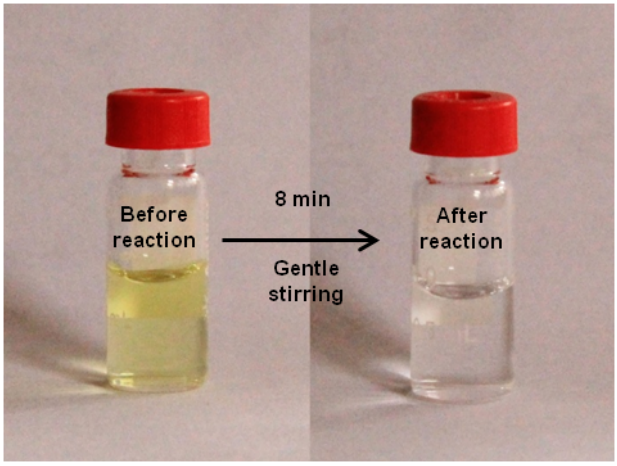

Fig. S4 Photograph of CoA-Au(I) CP (left: before formation, right: after formation). [CoA] = 4 mM, [Au(III)] = 2 mM.

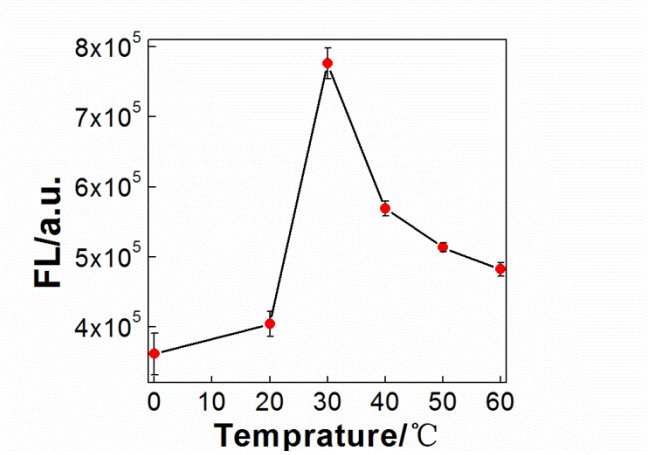

Fig. S5 The fluorescence intensity changes of SGII-stained CoA-Au(I) CP at 530 nm as a function of the reaction temperature. [CoA] = 100 μM, [Au(III)] = 50 μM.

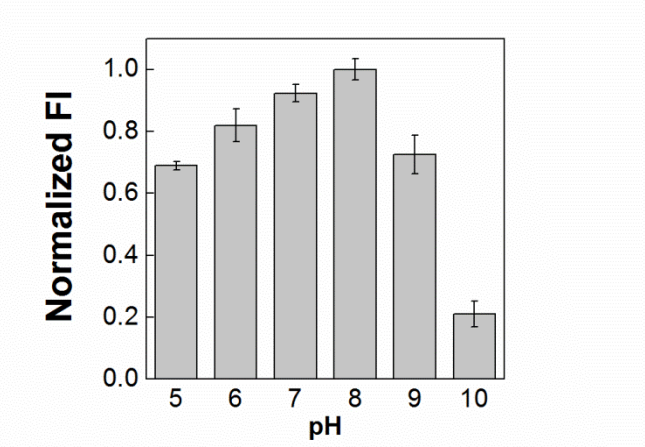

Fig. S6 Fluorescence intensity at 530 nm of the SGII-stained CoA-Au(I) CP as a function of the detection pH. [CoA] = 100 μM, [Au(III)] = 50 μM.

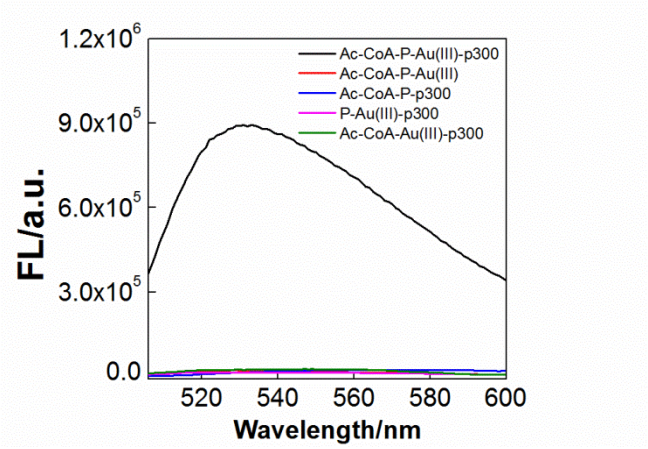

Fig. S7 Fluorescence detection of p300 activity by the CoA-Au(I) CP-based assay. The fluorescence response of the proposed assay to the full reaction mixture of Ac-CoA, peptide (P), Au(III), and p300 (black line); the same mixture without p300 (red line); without Au(III) (blue line); without Ac-CoA (pink line) or without peptide (green line). [p300] = 10 nM, [Ac-CoA] = 500 μM, [peptide] = 200 μM, [Au(III)] = 250 μM.

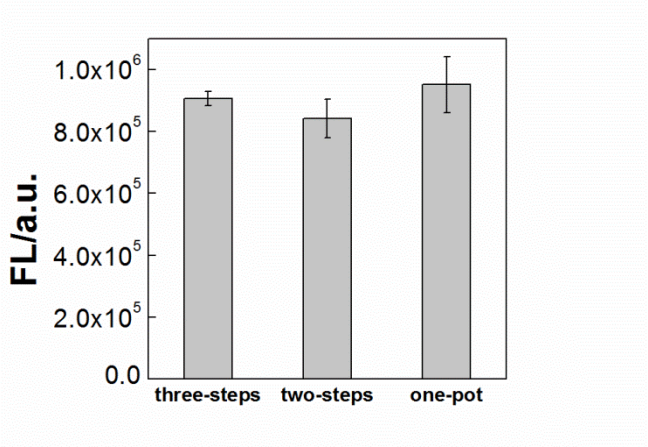

Fig. S8 Comparison of fluorescence signals measured by three different detection approaches**.** (i) three-steps approach: acetyl-CoA and substrate peptide were first treated by p300 for acetylation, then $HAuCl_4$ was introduced to synthesize CoA-Au(I) CP, followed by the addition of SGII for fluorescence signal-out; (ii) two-steps 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. [p300] = 10 nM, [Ac-CoA] = 500 μM, [peptide] = 200 μM, [Au(III)] = 250 μM.

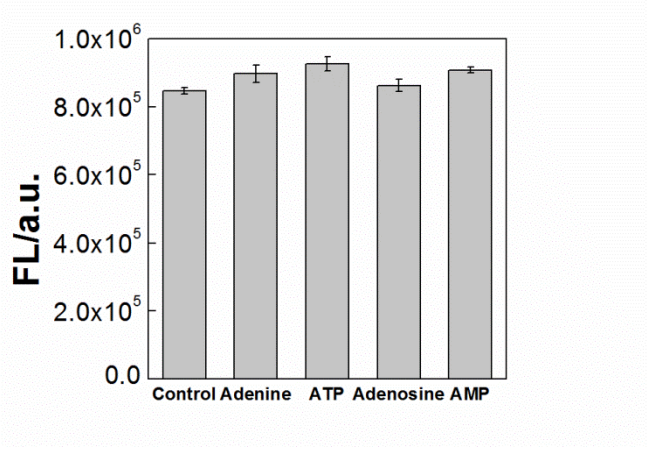

Fig. S9 Competition experiments to examine the fluorescence response of the proposed HAT assay in the presence of different co-existing adenine-containing compounds. Control: the fluorescence response of the proposed HAT assay in the absence of co-existing adenine-containing compound. [p300] = 100 nM, [Ac-CoA] = 500 μM, [peptide] = 200 μM, [Au(III)] = 250 μM, and [adenine-containing compounds] = 100 μM.

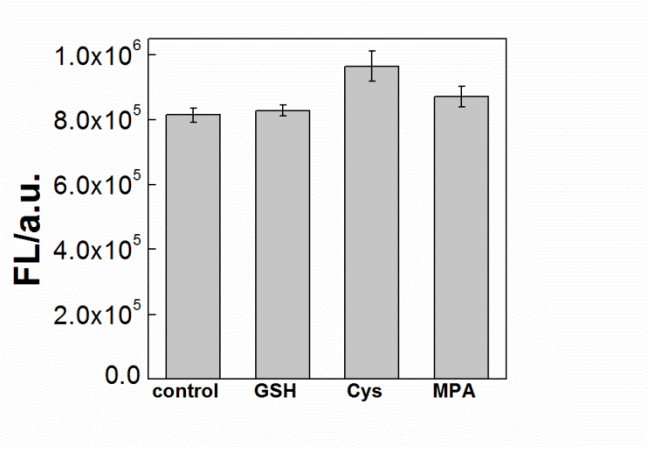

Fig. S10 Competition experiments to examine the fluorescence response of the proposed HAT assay in the presence of different co-existing thiol-containing compounds. Control: the fluorescence response of the proposed HAT assay in the absence of co-existing thiol-containing compound. [p300] = 100 nM, [Ac-CoA] = 500 μM, [peptide] = 200 μM, [Au(III)] = 250 μM, and [thiol-containing compounds] = 100 μM.

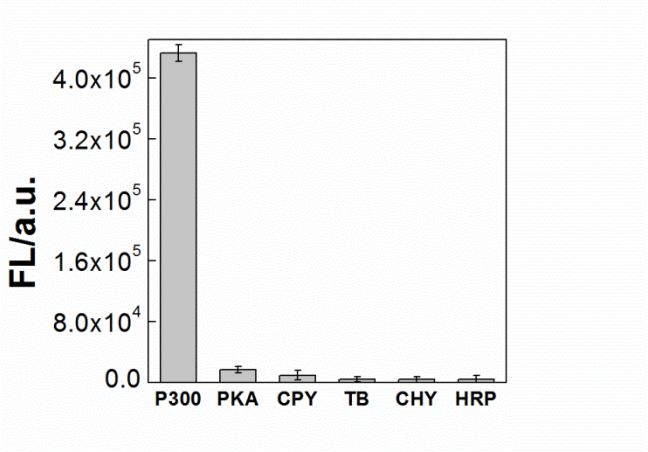

Fig. S11 Selectivity of the proposed HAT assay to p300. PKA: Protein kinase A, CPY: carboxypeptidase Y, TB: thrombin, CHY: chymotrypsin, and HRP: horseradish peroxidase. The concentration of each enzyme is 10 nM.

Table S1 Comparison of CoA-Au(I) CP based HAT assay with other previously-reported HAT assays.

Detection method	HAT	Antibody-free	Label-free	Detection steps	Detection limit	Detection range
FRET assay based on quantum-dots[1]	p300	No	No	2	0.5 nM[c]	0.5-100 nM
Colorimetric assay based on gold nanoparticles[2]	PCAF[a]	No	Yes	2	0.5 nM	2-200[b] nM
FRET assay based on donor and acceptor fluorophores[3]	p300	Yes	No	1	0.1 μM[c]	0.1-2.58 μM
Fluorescent assay based on sulfhydryl-sensitive dye[4]	PCAF[a]	Yes	No	2	--	0-200 nM
Our work base on nucleic acid-mimicking coordination polymer	p300	Yes	Yes	1	0.5 nM	0.2-100 nM[b]

[a] Histone acetyltransferase PCAF

[b] The linear range

[c] LDC: The lowest detectable concentration

References

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