Electronic Supplementary Information

Simultaneous single-molecule detection of acetyltransferase and crotonyltransferase activities of histone acetylation writer p300

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EXPERIMENTAL SECTION

Materials.

Peptide substrate (biotin-ARTKQTARKSTGGKAPRKQLATKAAR) was obtained from the Chinese Peptide Co.Ltd. (Zhengjiang, China). Recombinant GST-purified p300 HAT domain (p300), acetyl coenzyme A (AcCoA), crotonyl coenzyme A (CrCoA), immunoglobulin G (IgG) and anacradic acid were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Bovine serum albumin (BSA), cAMP-dependent protein kinase (PKA) and protein phosphatase 2A (PP2A) were purchased from New England Biolabs (Ipswich, MA, USA). 10% SDS was purchased from Life Technoiogies (Garlsbad, CA, USA). Histone deacetylase 1 (HDAC 1) was obtained from Cayman Chemical Company (Ann Arbor, Michigan, USA). 1× PBS (pH 7.4) was purchased from Solarbio Bioscience & Technology Co. Ltd. (Beijing, China). The streptavidin-coated magnetic beads (MBs) were obtained from Invitrogen (California, CA, USA). Anti-histone H3 (acetyl K18) antibody, anti-histone H3 (crotonyl K18) antibody, DyLight 488 conjugation kit and DyLight 650 conjugation kit were purchased from Abcam (Cambridge, MA). Human cervical carcinoma cell line (HeLa cells) was purchased from Cell Bank of Chinese Academy of Sciences (Shanghai, China).

In vitro HAT / HCT Activity Assays.

For in vitro HAT and HCT activities assays, various concentrations of p300 were incubated with 100 μM peptide substrate, 250 μM AcCoA or CrCoA, and 1× p300 reaction buffer (50 mM Tris–HCl, 1 mM DTT, 0.1 mM EDTA, 10 % glycerin, pH 8.5) in a total volume of 20 μL at 30 ºC for 2 h, followed by termination by incubation at 95 ℃ for 10 min. Then 0.5 μL of reaction products was incubated with 25 μL of streptavidin-coated magnetic beads (10 mg/mL) and 14.5 μL of 1×PBS (pH

7.4) on a roller mixer at room temperature for 30 min. To avoid the nonspecific binding, the mixture was blocked by 4 % BSA (w/v) on a roller mixer at room temperature for 20 min. Subsequently, the mixture was washed with 1×PBS (pH 7.4) for five times, followed by resuspending in 40 μL of 1×PBS. Then 1 μL of DyLight 488-conjugated anti-histone H3 (acetyl K18) antibody and 1 μL of DyLight 650-conjugated anti-histone H3 (crotonyl K18) antibody were added to the reaction mixture, and incubated for 30 min at the room temperature under dark in PBS buffer containing 0.1 % (w/v) BSA. The labeled peptides were washed and separated in a magnetic field, and resuspended in 100 μL of 0.01% SDS. Then the resuspended mixture was reacted at 95 °C for 10 min, and the supernatant solution was separated from the MBs on a preheated magnetic shelf. The supernatant was subjected to fluorescence measurement.

We measured the fluorescence intensity of reaction products using FLS1000 (Edinburgh Instruments, UK). The DyLight 488 fluorescence was measured at the excitation wavelength of 480 nm and the fluorescence intensity at 520 nm was used for quantitative analysis of HAT activity of p300. The DyLight 650 fluorescence was measured at the excitation wavelength of 635 nm and the fluorescence intensity at 675 nm was used for quantitative analysis of HCT activity of p300. The emission spectra were recorded over the wavelength range of 500 – 750 nm for DyLight 488 and 650 – 750 nm for DyLight 650. The excitation and emission slit widths are 6 nm and 4 nm, respectively.

Single-molecule detection and data analysis.

For single-molecule detection, the reaction products were diluted 50-fold with the imaging buffer (67 mM Glycine-KOH, 2.5 mM $MgCl_2$, 5 μg/mL BSA, 1 mg/mL Trolox, pH 9.4). The 10 μL of

sample was spread on a glass coverslip for imaging. The images of single molecules were acquired by total internal reflection fluorescence (TIRF) microscopy (Nikon, Ti-E, Japan). The 488 nm and 640 nm lasers were used to excite DyLight 488 and DyLight 650 fluorescent molecules, respectively. The photons of DyLight 488 and DyLight 650 were collected by using an oil immersion 100× objective. The photons from DyLight 488 and DyLight 650 were collected by camera (Photometrics, Evolve 512). For data analysis, regions of interest of 500 × 500 pixels were selected for counting DyLight 488 and DyLight 650 fluorescent molecules by using ImageJ software. The numbers of DyLight 488 and DyLight 650 fluorescent molecules were the average of 10 frames, respectively. The single molecule photobleaching experiment was carried out as previously described.[1, 2] In brief, the diluted reaction mixture was continuously excited by either a 488 nm laser (20 mW) for DyLight 488 or a 640 nm laser (20 mW) for DyLight 650, and the live images were recorded at a high-frequency with an exposure time of 100 ms. Three hundred frames were used for data analysis. All the measurements were performed at room temperature.

Inhibition assay.

We investigated the effects of anacardic acid (a typical HAT inhibitor) on the HAT and HCT activities of p300. After incubating different-concentration inhibitors with 1 nM p300 and 250 μM of AcCoA / CrCoA, the measurements of DyLight 488/DyLight 650 counts follow same procedure described above. The relative activity (*RA*) of HAT was calculated based on equation 1.

$$RA = \frac{C_i}{C_t} \times 100\ \% = 10^{(N_i - N_t)/18.13} \times 100\ \% \qquad (1)$$

where N_t represents the Dylight488 counts in the presence of 1 nM p300, and N_i represents the Dylight 488 counts in the presence of 1 nM p300 + anacardic acid. C_i and C_t were obtained according

to the linear correlation equation (Fig. 3A), respectively. The IC_{50} value of inhibitor was obtained from the curve-fitting equation.

$$N_t = 24.74 + 18.13log_{10}C_t \quad (2)$$

$$N_i = 24.74 + 18.13log_{10}C_i \quad (3)$$

The relative activity (RA) of HCT was calculated based on equation 4.

$$RA = \frac{C_i}{C_t} \times 100\ \% = 10^{(N_i - N_t)/43.66} \times 100\ \% \quad (4)$$

where N_t represents the DyLight 650 counts in the presence of 1 nM p300, and N_i represents the DyLight 650 counts in the presence of 1 nM p300 + anacardic acid. C_i and C_t were obtained according to the linear correlation equation (Fig. 3B), respectively. The IC_{50} value of inhibitor was obtained from the curve-fitting equation.

$$N_t = 189.62 + 43.66\ log_{10}C_t \quad (5)$$

$$N_i = 189.62 + 43.66\ log_{10}C_i \quad (6)$$

Cell culture and preparation of cell extracts.

HeLa cells was cultured with 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin-streptomycin (Invitrogen, USA) in Dulbecco's modfied Eagle's medium (DMEM). The cells were cultured at 37 °C in a humidified atmosphere containing 5% CO_2. The 1×10^6 cells were collected and washed twice with 1× PBS (pH 7.4). The number of cells was measured by Countstar automated cell counter (IC1000, Wilmington, DE, USA). The nuclear extracts of HeLa cells were prepared by using the nuclear extract kit (ActiveMotif, Carlsbad, CA, USA) according to the manufacturer's protocol. The obtained supernatant was subjected to fluorescence measurement.

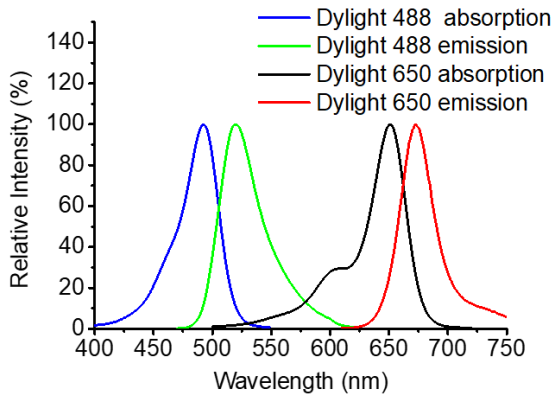

Fig. S1 Normalized absorption and emission spectra of Dylight 488 and Dylight 650. Blue line, absorption spectrum of Dylight 488; green line, emission spectrum of Dylight 488; black line, absorption spectrum of Dylight 650; red line, emission spectrum of Dylight 650.

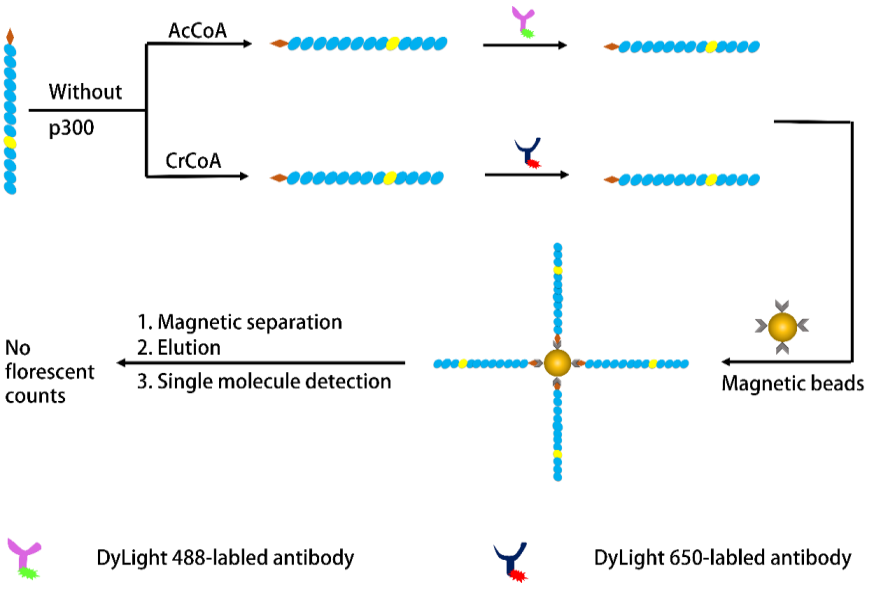

Fig. S2 Schematic illustration of single-molecule detection in the absence of histone acetylation writer p300.

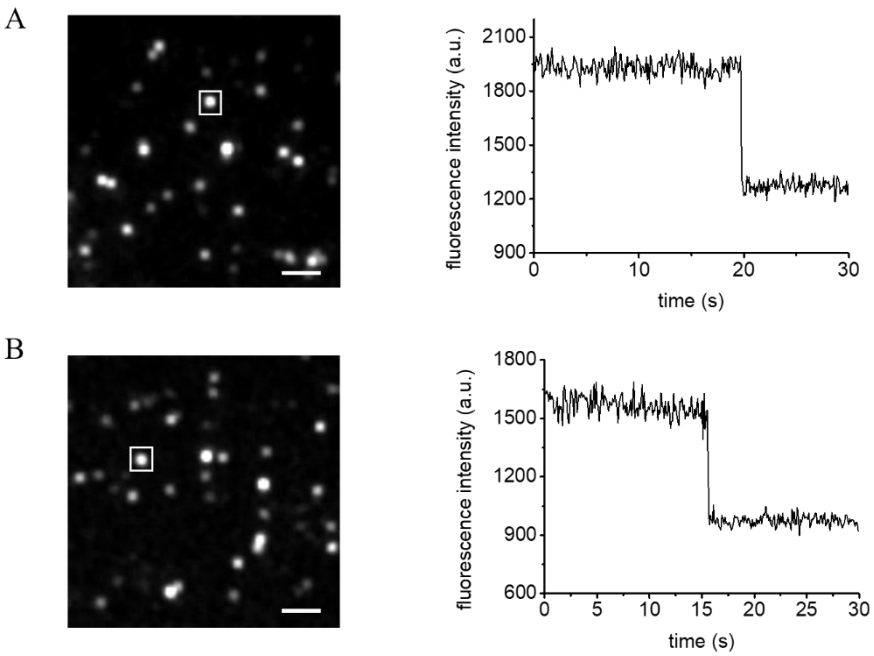

Fig. S3 Intensity traces over time showing one photobleaching step for individual DyLight 488 (A) and DyLight 650 (B) fluorescence spots. The fluorescence images prior to photobleaching process are shown in the left panel. The scale bar is 1 μm.

Optimization of Experimental Conditions.

To achieve the best assay performance, we optimized reaction time of p300. As shown in Fig. S4A, DyLight 488 counts improve with reaction time from 0.5 to 2.5 h and reaches a plateau at 2 h due to either the complete loss of HAT activity of p300 or the consumption of all available substrates by p300. Similarly, DyLight 650 counts improve with reaction time from 0.5 to 2.5 h and reaches a plateau at 2 h. Thus, the incubation time of 2 h is used in the subsequent research. We further optimized the amount of magnetic beads by measuring the DyLight 488/ DyLight 650 counts in response to the added magnetic beads. As shown in Fig. S4B, the DyLight 488 counts reach the highest value at 250 μg, followed by the decrease beyond the amount of 250 μg. Similarly, the DyLight 650 counts reach the highest value at 250 μg, followed by the decrease beyond the amount

of 250 μg (Fig. S4B). Therefore, 250 μg of magnetic beads is used for the simultaneous detection of dual enzymatic activities of p300.

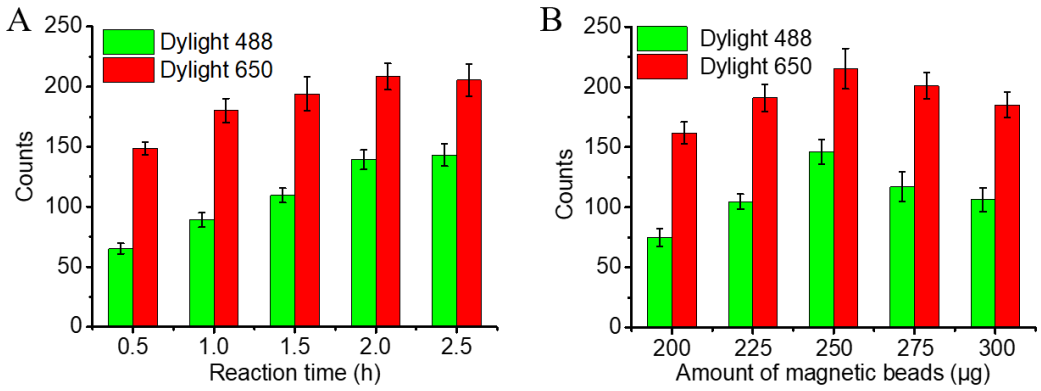

Fig. S4 (A)Variance of the counts of DyLight 488 (green) and DyLight 650 (red) with reaction time. (B)Variance of the counts of DyLight 488 (green) and DyLight 650 (red) with different amounts of magnetic beads. The p300 concentration is 125 nM. Error bars show the standard deviation of three experiments.

Conversion Equation.

We measured the DyLight 488/ DyLight 650 counts in the experiments. To convert the DyLight 488 counts to the concentration of AcCoA, we performed a series of experiments using varying concentrations of AcCoA, high-concentration enzyme and high-concentration peptide to ensure the complete proceeding of HAT reaction.[3] The data were fitted with a linear equation (Fig. S5A). The consumed AcCoA may be calculated from the standard curves. To convert the DyLight 650 counts to the concentration of CrCoA, we performed a series of experiments using varying concentrations of CrCoA, high-concentration enzyme and high-concentration peptide to ensure the complete proceeding of HCT reaction. The data were fitted with a linear equation (Fig. S5B). The consumed CrCoA may be calculated from the standard curves.

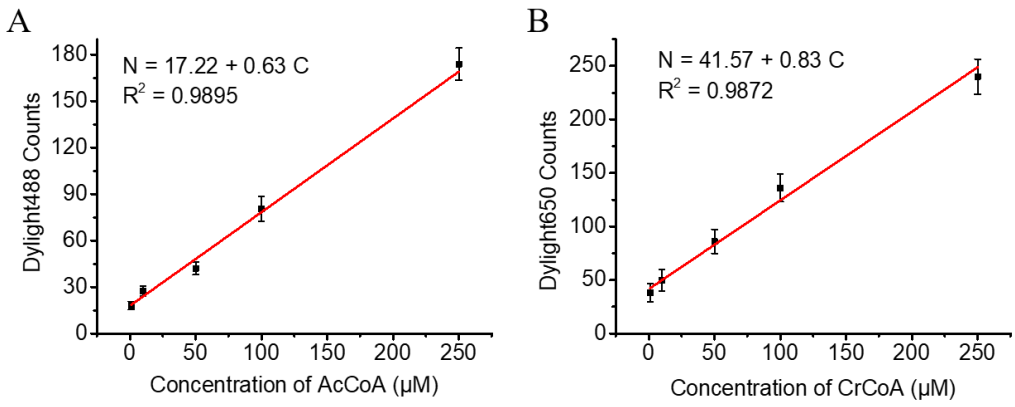

Fig. S5 (A) Variance of the Dylight 488 counts with different concentrations of AcCoA. (B) Variance of the Dylight 650 counts with different concentrations of CrCoA. The p300 concentration is 125 nM. Error bars show the standard deviation of three experiments.

Measurement of HAT and HCT activities of p300 in the spiked HeLa cell extracts.

The 20 μL of sample containing 5 μL of HeLa cell extracts, 125 nM p300, 100 μM peptide substrate, 250 μM AcCoA, 250 μM CrCoA, and 1× p300 reaction buffer was incubated at 30 ºC for 2 h. Then the subsequent experiments were carried out according to the procedures described above. We further measured the HAT and HCT activities of p300 in the spiked crude cell extracts after incubation with the inhibitor. The relative enzyme activity is determined according to $A(\%) = 100 \times (N_i/N_0)$, where N_i is the measured counts in the presence of anacardic acid, and N_0 is the measured counts in the control sample without anacardic acid. As shown in Fig. S6, anacardic acid induces the decrease in the relative activity of HAT of p300, while the relative activity of HCT of p300 remains unchanged.

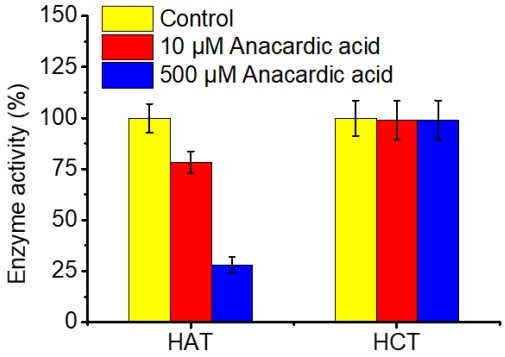

Fig. S6 Simultaneous detection of both HAT and HCT activities in the spiked HeLa cell extracts. 0 μM (yellow column), 10 μM (red column) and 500 μM (blue column) anacardic acid were used in the experiments. The p300 concentration is 125 nM. Error bars show the standard deviation of three experiments.

Table S1. Comparison of the proposed method with the reported histone modification enzyme (HMEs) assays.

Detection method	Target	Linear range	Detection limitation	Reference
Fluorescent assay	p300 HAT	0.5 – 100 nM	0.2 nM	4
Luminescent biosensor	p300 HAT	0.2 – 100 nM	0.05 nM	5
Fluorometric biosensor	p300 HAT	0.1 – 120 nM	0.05 nM	6
	histone deacetylases (HDAC)	1 – 450 nM	1 nM	
Electrochemiluminescent immunosensor	p300 HAT	0.006 – 60 nM	0.0029 nM	7
Electrogenerated chemiluminescent biosensor	p300 HAT	0.1 – 100 nM	0.074 nM	8
Colorimetric assay	SET 7/9 histone methyltransferase	1 – 200 nM	0.2 nM	9
	pCAF HAT	2 – 200 nM	0.5 nM	
Surface enhanced Raman scattering (SERS) method	LSD1 histone demethylases	2 – 200 nM	0.6 nM	10
Single-molecule detection	p300 HAT	3×10^{-4} – 10 nM	1.75×10^{-4} nM	This work
	p300 HCT	3×10^{-4} – 1 nM	6.56×10^{-5} nM	

References

1. X. Shi, Y. He, W. Gao, X. Liu, Z. Ye, H. Liu and L. Xiao, Anal. Chem., 2018, 90, 3661-3665.

2. Y. Li, Y. Yang and C.-y. Zhang, Anal. Chem., 2018, 90, 13007-13012.

3. I. L. Medintz, A. R. Clapp, F. M. Brunel, T. Tiefenbrunn, H. T. Uyeda, E. L. Chang, J. R. Deschamps, P. E. Dawson and H. Mattoussi, *Nat. Mater.*, 2006, **5**, 581-589.

4. S. Chen, Y. Li, Y. Hu, Y. Han, Y. Huang, Z. Nie and S. Yao, *Chem. Commun.*, 2015, **51**, 4469-4472.

5. Y. Han, H. Li, Y. Hu, P. Li, H. Wang, Z. Nie and S. Yao, *Anal. Chem.*, 2015, **87**, 9179-9185.

6. H. Wang, Y. Li, K. Zhao, S. Chen, Q. Wang, B. Lin, Z. Nie and S. Yao, *Biosens. Bioelectron.*, 2017, **91**, 400-407.

7. Q. Zhang, D. Hu, T. Zhan, Y. Hu, Z. Guo and S. Wang, *Sens. Actuator B-Chem.*, 2020, **316**, 128165.

8. Y. Zou, Z. Wang, H. Zhang and Y. Liu, *Biosens. Bioelectron.*, 2018, **122**, 205-210.

9. Z. Zhen, L.-J. Tang, H. Long and J.-H. Jiang, *Anal. Chem.*, 2012, **84**, 3614-3620.

10. Y. Wang, X. Deng, J. Liu, H. Tang and J. Jiang, *Chem. Commun.*, 2013, **49**, 8489-8491.