Electronic Supplementary Information

Multiplex Detection of Histone-Modifying Enzymes by Total Internal Reflection Fluorescence-based Single-Molecule Detection

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EXPERIMENTAL SECTION

Materials. The recombinant enzymes including GcN5 (residues 2-837), G9a (residues 785-1210), TIP60 (residues 2-513) and PRMT1 (residues 2-371) were purchased from Cayman Chemical (Ann Arbor, MI, USA). The N-terminal biotinylated peptide substrate (biotin-ART KQT ARK STG GKA PRK QLA) and the non-specific biotinylated peptide (biotin-TQL KGT THK CLR SKC LKK RAS) were synthesized and purified by Ontores Biotechnologies (Hangzhou, Zhejiang, China). The anti-acetyl lysine (H3-K14) and anti-methyl lysine (H3-K9) were obtained from Cell Signaling Technology (Beverly, MA, USA) and conjugated with Alexa Fluor 488 and Alexa Fluor 647 (Invitrogen, Carlsbad, CA, USA), respectively, according to the manufacturer's instructions. The recombinant PKA and S-adenosylmethionine (SAM) were purchased from New England BioLabs (Beverly, MA, USA). The streptavidin-coated magnetic beads (Dynabeads® M-280 Streptavidin, Dynal) were obtained from Invitrogen (Carlsbad, CA, USA). Acetyl-coenzyme A (AcCoA), butyrolactone MB-3 and BIX-01294 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Enzyme Reaction and Magnetic Separation. The enzymes at various concentrations were added to 10 μL of reaction buffer (50 mM Tris-HCl, pH 8.5, 1 mM DTT, 0.1 mM EDTA, 0.01% Tween-20) containing 50 μM substrate peptide, and the reaction was initiated with the addition of 80 μM SAM and 100 μM AcCoA. The mixture was incubated at 35 °C for 30 min. To avoid the nonspecific binding, both the reaction mixture and the magnetic beads were blocked by 1% BSA (w/v) for 20 min. Subsequently, 1 μL of reaction mixture, 50 μL of magnetic beads, 0.1 μL of Alexa Fluor 488-conjugated anti-acetyl lysine antibody and 0.1 μL of Alexa Fluor 647-conjugated

anti-methyl lysine antibody were mixed and incubated for 30 min with gentle rotation in PBS buffer containing 0.1% (w/v) BSA. Finally, the labeled peptides were separated in a magnetic field as described previously,[1] followed by elution from the beads using deionized water at 70 °C.[2]

Single-Molecule Detection. To obtain the images of single molecules by total internal reflection fluorescence (TIRF) microscopy, we employed an inverted Olympus IX-71 microscope (Olympus, Tokyo, Japan) equipped with a UAPON 100× TIRF objective (1.49 NA, Olympus). A sapphire 488 nm laser (50 mW, Coherent, USA) was used to excite Alexa Fluor 488, and a cube 640 nm laser (100 mW, Coherent, USA) was used to excite Alexa Fluor 647. Two long-pass edge filters of BLP01-488R-25 and BLP01-635R-25 (Semrock, USA) were placed between the objective lens and the EMCCD (DU-897, Andor, UK) to collect the emitted photons. The setup of TIRF instrument is shown in Fig. S1. For each sample, 30 μL of elution solution was spread on a polylysine-covered glass coverslip and imaged with an exposure time of 100 ms. An image series of 10 frames on 10 different locations were acquired from a single slide. All the images were acquired by Micro-manager 1.4 software (http://www.micro-manager.org), and were quantitatively analyzed with the *Image J* software (NIH, Bethesda, MD, USA) as described previously.[3] The size of particles was set at 2-10 pixels to reduce false positive signals generated from noises. For data analysis, a region of interest (ROI) with 200 × 200 pixels (32 × 32 μm^2) was selected for single molecule counting. For each sample, the fluorescent spots in 10 frames were counted and summed up, and the number of fluorescent molecules counted (*N*) is the average number in 10 frames. All experiments were done in triplicate and repeated at least twice to prove their reproducibility.

Cell Culture and Preparation of Cell Extracts. Hela cells were cultured in Dulbecco's modified Eagles medium (DMEM) with 10% fetal bovine serum (FBS) and 50 U/mL of penicillin plus 50 μg/mL streptomycin in a 100% humidified chamber containing 5% CO_2 at 37 °C. The nuclear extracts were prepared using a CelLytic NuCLEAR Extraction kit (Sigma, St. Louis, MO, USA) according to the manufacturer's instructions, and the samples equivalent to 1×10^5 cells were used for enzyme assay.

SUPPLEMENTARY RESULTS

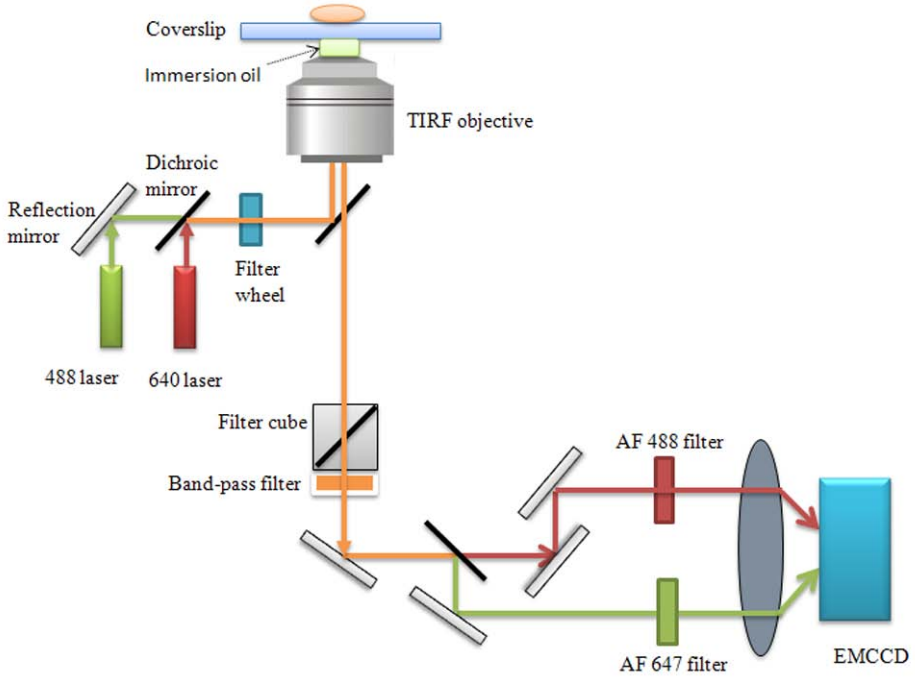

Fig. S1 Setup of TIRF instrument.

In current design, Alexa Fluor 488 is used to label the anti-acetyl lysine (H3-K14) and Alexa Fluor 647 is used to label the anti-methyl lysine (H3-K9). The maximum emission wavelength is 519 nm for Alexa Fluor 488 and 665 nm for Alexa Fluor 647, and there is no spectral overlap between the emission of Alexa Fluor 488 and that of Alexa Fluor 647 (Fig. S2). In addition, Alexa Fluor 488 can only be excited by the 488 nm laser, and Alexa Fluor 647 can only be excited by the 640 nm laser. Therefore, there is no cross-talk between Alexa Fluor 488 and Alexa Fluor 647. These results clearly demonstrate that the fluorescent pair of Alexa Fluor 488/Alexa Fluor 647 is suitable for simultaneous detection of GcN5 and G9a.

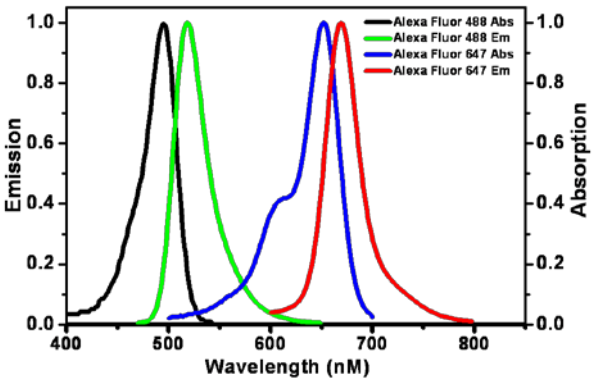

Fig. S2 The normalized absorption and emission spectra of Alexa Fluor 488 and Alexa Fluor 647. Black line, absorption spectrum of Alexa Fluor 488; green line, emission spectrum of Alexa Fluor 488; blue line, absorption spectrum of Alexa Fluor 647; red line, emission spectrum of Alexa Fluor 647.

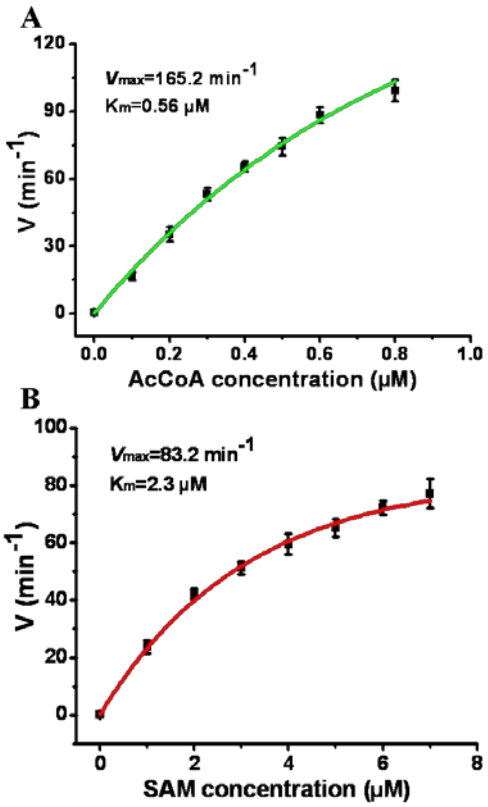

Fig. S3 Kinetic analysis of GcN5 (A) and G9a (B). Error bars show the standard deviation of three experiments.

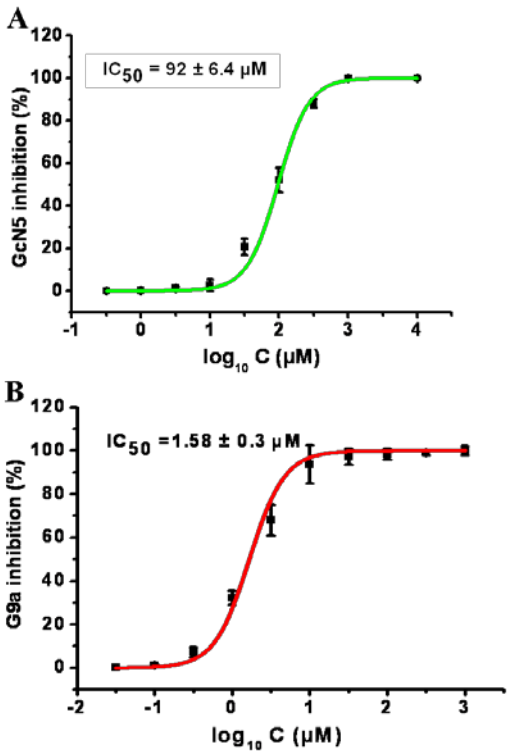

Fig. S4 (A) Inhibition of GcN5 by different-concentration MB-3. (B) Inhibition of G9a by different-concentration BIX-01284. Error bars show the standard deviation of three experiments.

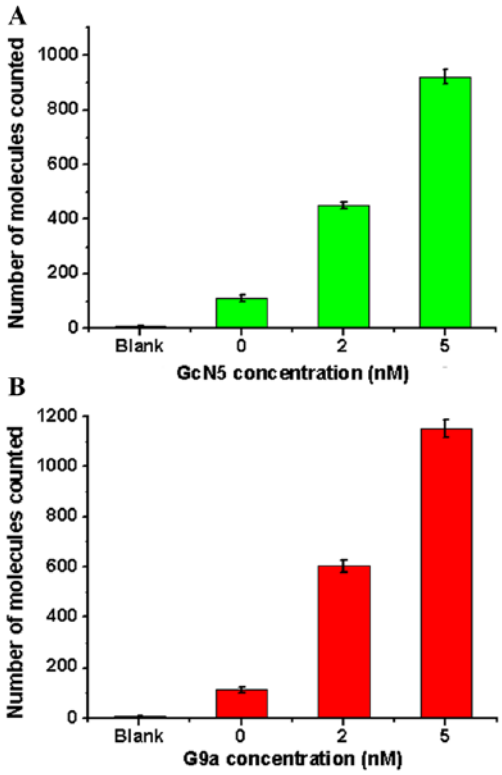

Fig. S5 Detection of GcN5 (A) and G9a (B) activities in crude cell extracts, respectively, after the addition of different-concentration GcN5 / G9a. The sample with only extract buffer was used as the blank. Error bars show the standard deviation of three experiments.

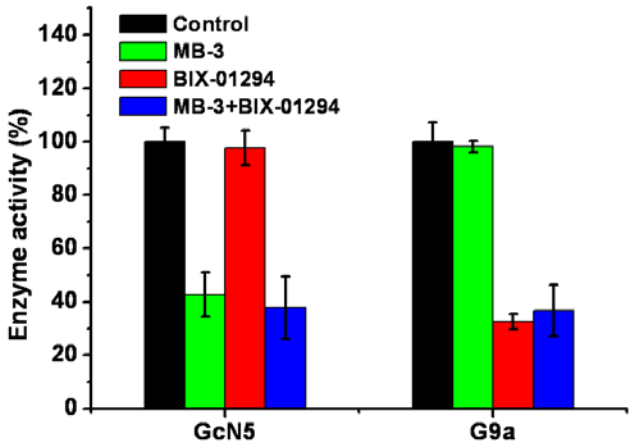

Fig. S6 Simultaneous detection of GcN5 and G9a activities in crude cell extracts. MB-3 (100 μM) is used as GcN5 inhibitor, and BIX-01294 (2 μM) is used as G9a inhibitor. Error bars show the standard deviation of three experiments.

REFERENCES

1 S. Girault, G. Chassaing, J. Blais, A. Brunot and G. Bolbach, *Anal. Chem.*, 1996, **68**, 2122.

2 A. Holmberg, A. Blomstergren, O. Nord, M. Lukacs, J. Lundeberg and M. Uhlén, *Electrophoresis*, 2005, **26**, 501.

3 H. M. Chan, L. S. Chan, R. N. S. Wong and H. W. Li, *Anal. Chem.*, 2010, **82**, 6911.