Supplementary Information

Ligand-Regulated Catalytic Activity in Fluorescent Gold Nanoclusters

*Wenxuan Yang,[1] Shenming Wang,[1] Xiaoyun Wei, Zhenzhen Feng,[2] Guangyong Qin,[1] Jian Mao,[1] Zheng Zhen,[1] Xintong Miao,[1] Yifeng Cheng,[1] Xiaojuan Wang,[1] Huang Fang,[1] and Hua He *[1]*

[1] State Key Laboratory of Heavy Oil Processing and College of Chemistry and Chemical Engineering, China University of Petroleum (East China), Qingdao 266580, China

Email: huahe@upc.edu.cn

[2] Technical Center of Qingdao Customs District, Qingdao 266500, China

EXPERIMENTAL METHODS

Synthesis of MPA-AuNCs

MPA-AuNCs were synthesized following established protocols.[1] An aqueous solution of $HAuCl_4 \cdot 4H_2O$ (20 mM, 0.25 mL) and mercaptopropionic acid (MPA) (5 mM, 2 mL) was added to 2.35 mL of water and stirred at room temperature for 5 minutes. Subsequently, 0.3 mL of NaOH solution (1 M) was added, followed by 0.1 mL of $NaBH_4$ solution, prepared by dissolving 43 mg of $NaBH_4$ in 10 mL of 0.2 M NaOH. The reaction was conducted in the dark and stirred at room temperature for 3 hours. The resulting MPA-AuNCs were then collected.

Synthesis of GSH-AuNCs

GSH-AuNCs were synthesized based on previously reported methods.[2] Briefly, a freshly prepared solution of $HAuCl_4 \cdot 4H_2O$ (20 mM, 500 μL) was mixed with glutathione (GSH) (20 mM, 1 mL), and the mixture was maintained at 70°C for 12 hours. After cooling, the initial GSH-AuNCs product was obtained. This product was then filtered using ultrafiltration tubes of 30 kDa and 3 kDa at 10,000 rpm, yielding gold clusters with a molecular weight range of 3 kDa to 30 kDa.

Synthesis of KCK-AuNCs

A freshly prepared KCK solution (20 mM, 0.5 mL) was mixed with TCEP (20 mM, 0.5 mL) and incubated in a dark water bath at 70°C for 15 minutes. A solution of $HAuCl_4 \cdot 4H_2O$ (20 mM, 500 μL) was then quickly added, the mixture was homogenized, and the reaction was continued in the dark at 70°C for another 10 minutes. NaOH solution (1.5 M, 50 μL) and $NaBH_4$ solution (0.1 M, 8 μL) were sequentially added to a glass vial and mixed thoroughly with a pipette. An additional 3.4 mL of ultrapure water was added to achieve a final concentration of 2 mmol/L of Au in the solution. The reaction was maintained at 70°C for 10 hours. The product was then filtered using

ultrafiltration tubes of 30 kDa and 3 kDa at 10,000 rpm, yielding gold clusters within a molecular weight range of 3 kDa to 30 kDa.

Synthesis of BSA-AuNCs

BSA-AuNCs were synthesized as previously described.[3] $HAuCl_4 \cdot 4H_2O$ (5 mL, 10 mM) was added to a BSA solution (5 mL, 50 mg/mL) at 37°C under vigorous stirring. After 2 minutes, 0.5 mL of NaOH solution (1 M) was added, and the mixture was incubated at 37°C for 12 hours. During this period, the solution color changed from light yellow to light brown, and finally to dark brown.

Characterizations of AuNCs

All optical characterizations were conducted at room temperature, approximately 25 °C. Mass spectra were recorded using an Autoflex III smartbeam MALDI-TOF mass spectrometer. UV-Vis absorption spectra were recorded on a Shimadzu UV-2450 spectrophotometer. Fluorescence spectra and lifetime were measured with a FS5 fluorescence spectrophotometer (UK). TEM images were captured using a JEM-2100 transmission electron microscope operating at 200 kV to examine the morphology of the AuNCs.

Single molecule fluorescence imaging

AuNCs were first immobilized on a cleaned coverslip, which was then adhered to a slide with two holes to form a reaction cell. The reactant solution was continuously supplied via a microfluidic syringe at a flow rate of 30 μL/min. Fluorescence was excited using a 532 nm laser on a Nikon total internal reflection fluorescence microscope (TIRFM). The emitted fluorescence was collected using a 100× objective (NA = 1.49) equipped with an integrated 1.5× magnification changer lens, a 570 nm dichroic mirror, and a 593/40 nm band-pass filter. Detection was performed using an Electron Multiplying Charge-Coupled Device (EMCCD; iXon 897, Andor, 16 μm/pixel). The EMCCD's gain and read-out rate were set at 300 and 10 MHz, respectively. Fluorescence

intensity was converted into photon counts using the electron/Analog Digital Units (ADU) conversion factor (DCF), EM gain, and quantum efficiency (QE). The relationship used was: photons = ADU × DCF / (QE × gain), where DCF was 12.02 and QE was approximately 92% for the 600-750 nm fluorescence range. Data was analyzed using a custom MATLAB program, which extracted single molecule signals from a 7 × 7-pixel region centered around the peak intensity. Fluorescence blinking events were identified by isolating signals that exceeded four times the standard deviation of the background fluorescence trajectory fluctuations.

Movie S1

Movie S1. Blinking from single particle catalysis

Figures S1-S14

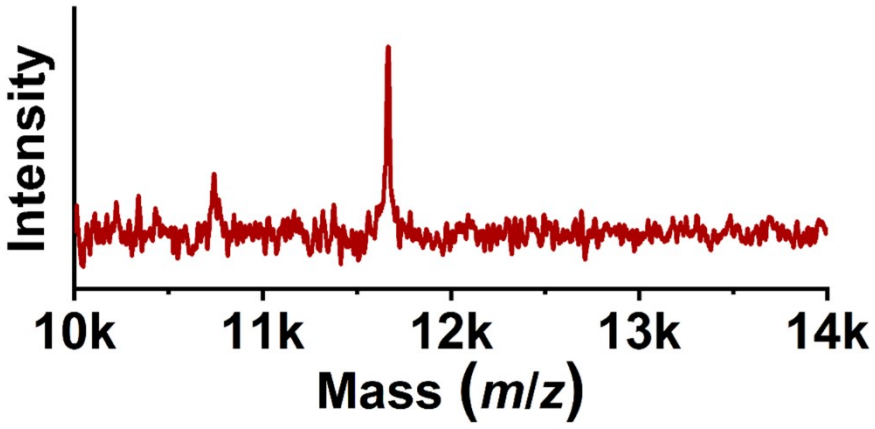

Figure S1. Mass spectrometry of KCK-AuNCs, showing a molecular weight consistent with $Au_{25}(KCK)_{18}$.

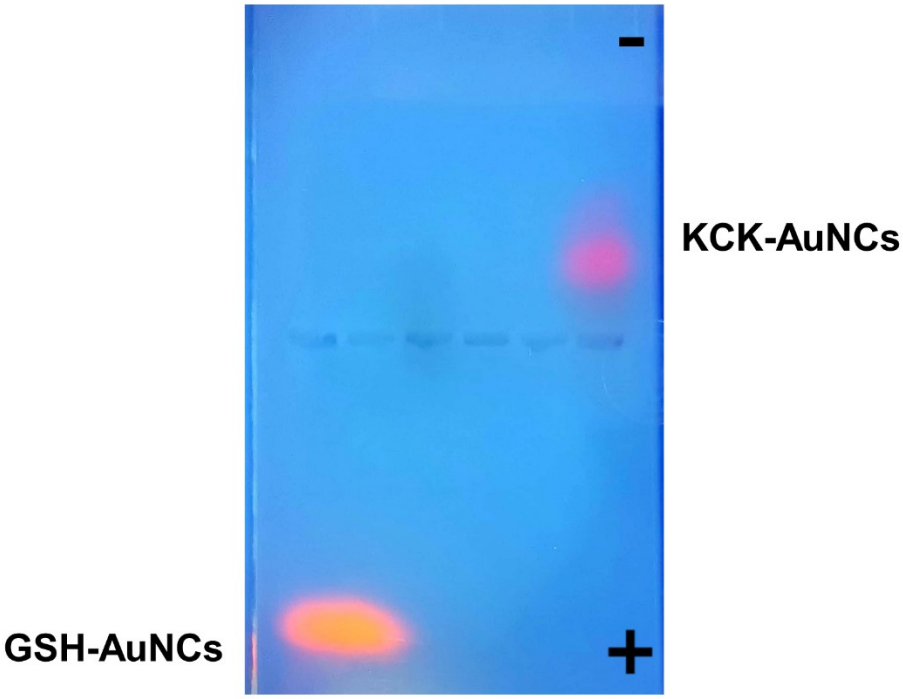

Figure S2. Electrophoresis photo of GSH-AuNCs and KCK-AuNCs.

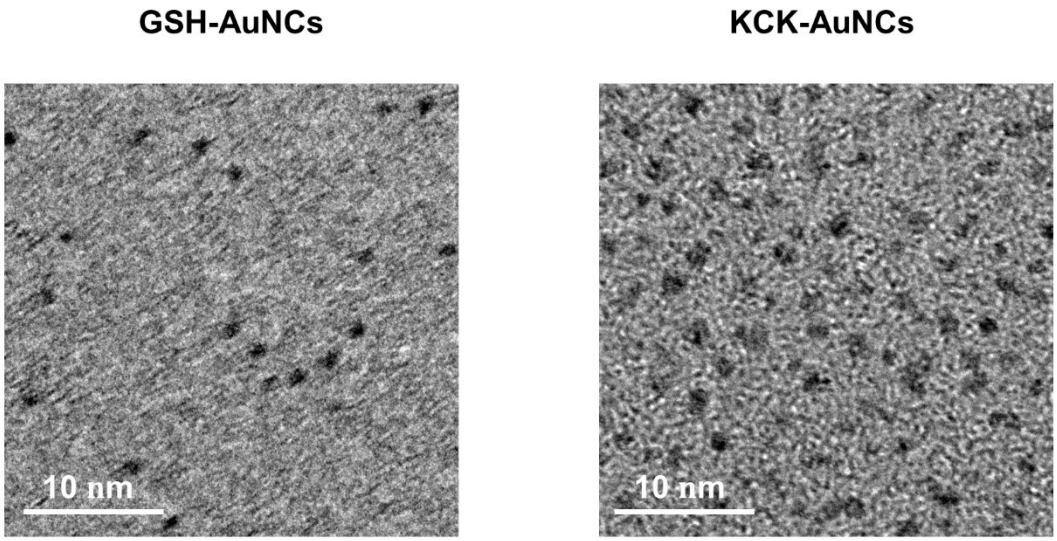

Figure S3. TEM images of (a) GSH-AuNCs and (b) KCK-AuNCs, showing their average size to be around 2 nm.

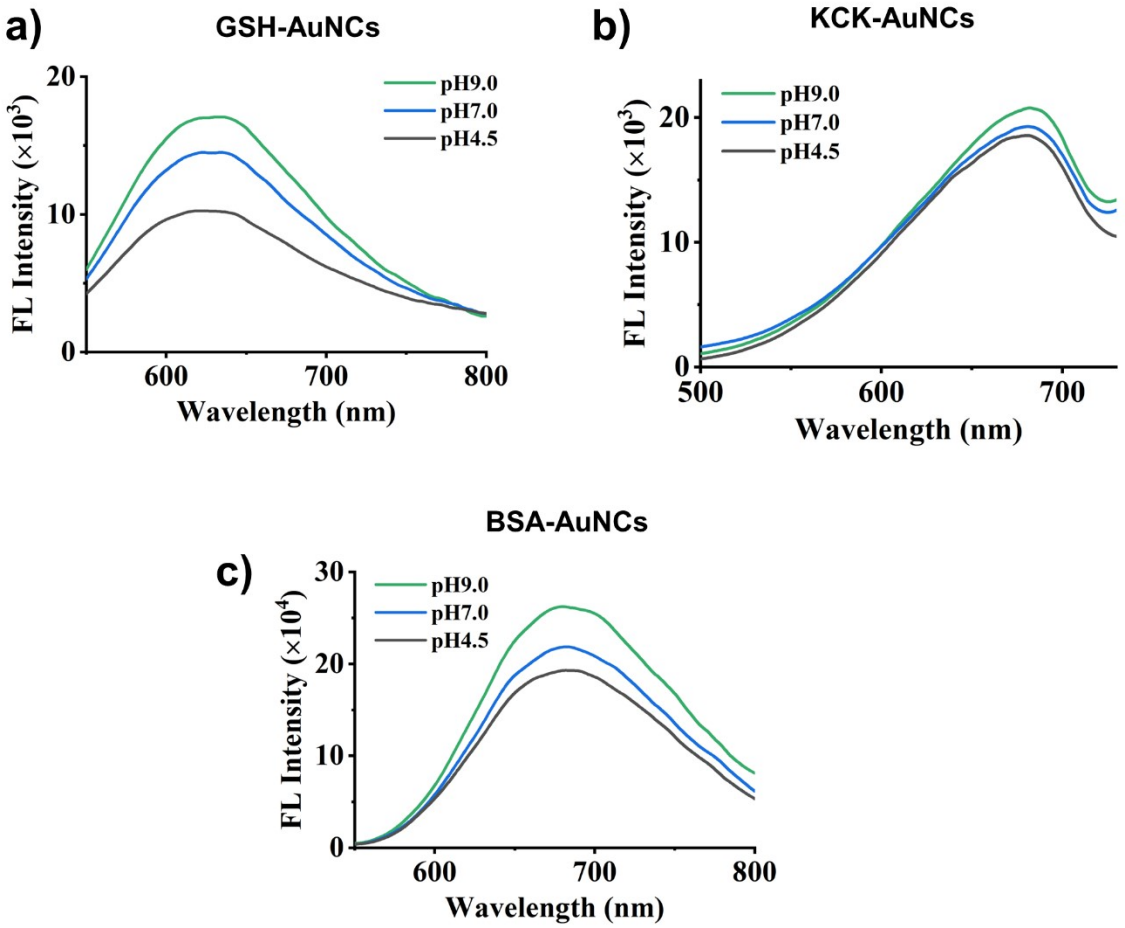

Figure S4. Effect of pH on the fluorescence of AuNCs. (a) GSH-AuNCs, (b) KCK-AuNCs, and (c) BSA-AuNCs.

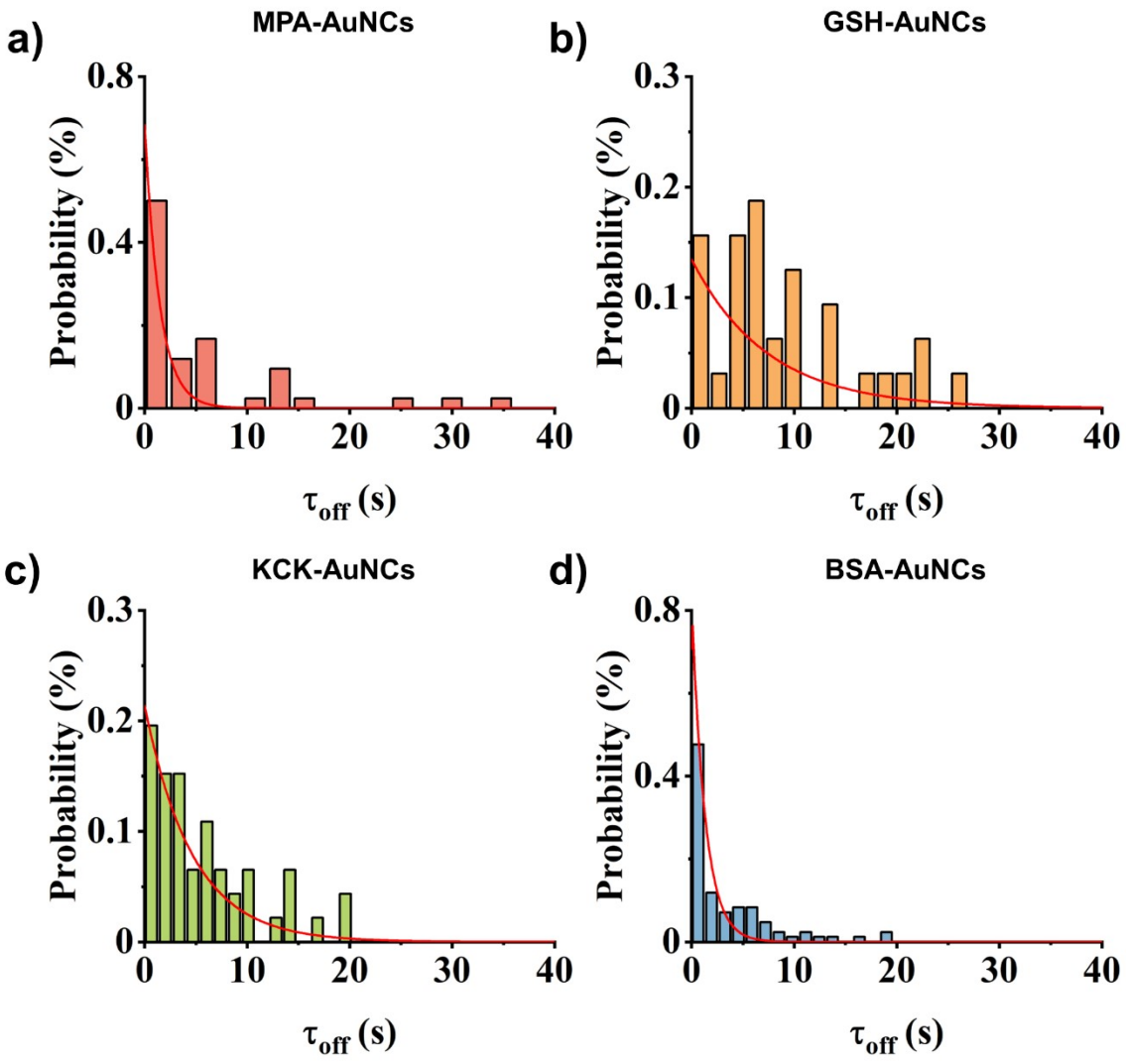

Figure S5. Distribution of off-time during the single-molecule catalytic process for the four AuNCs. (a) MPA-AuNCs, (b) GSH-AuNCs, (c) KCK-AuNCs, and (d) BSA-AuNCs.

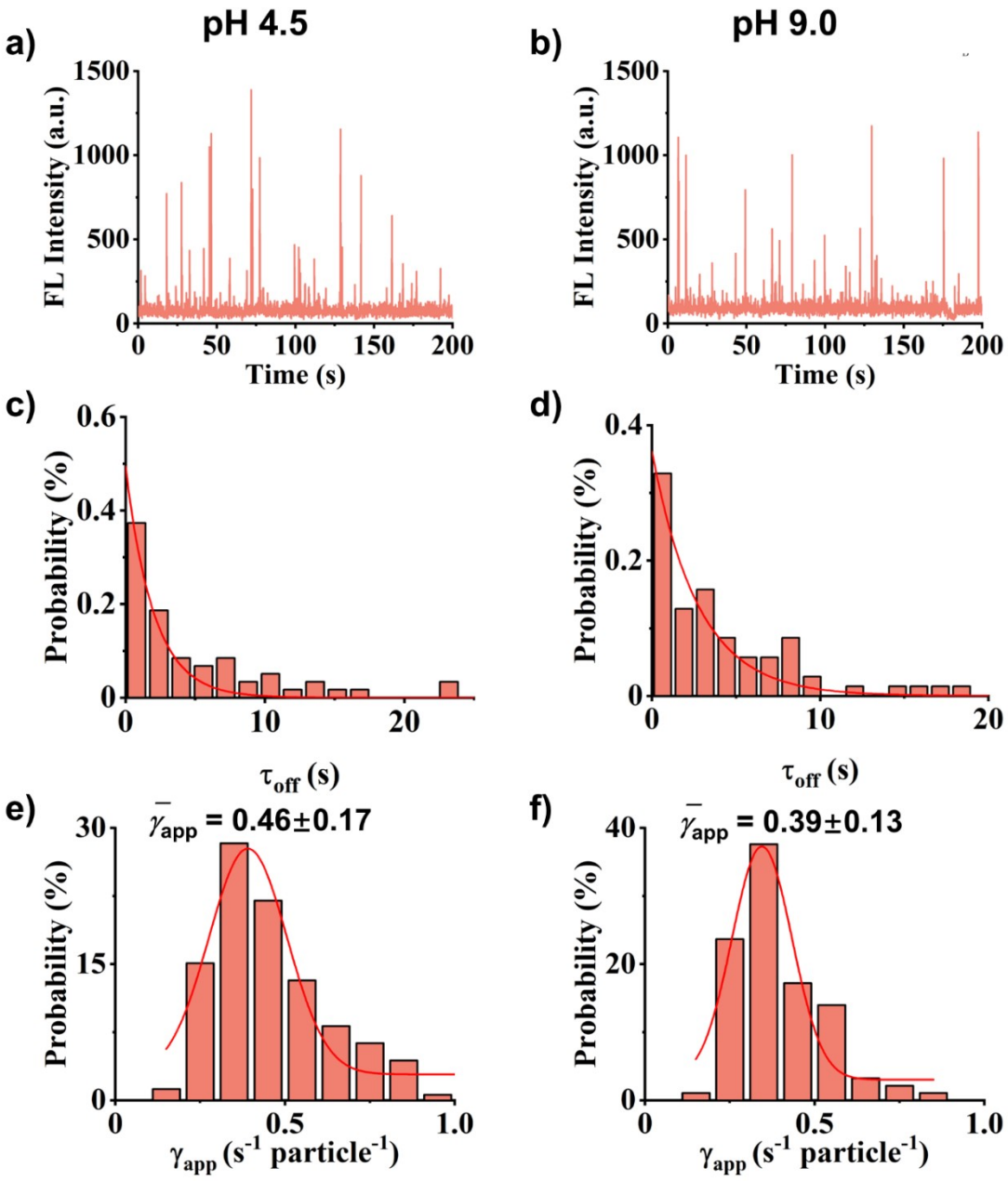

Figure S6. (a-b) Fluorescence intensity-time trajectories of individual MPA-AuNCs at pH 4.5 and pH 9.0, respectively. (c-d) Off-time distribution of MPA-AuNCs at pH 4.5 and pH 9.0, respectively. (e-f) Distribution of γ_{app} for MPA-AuNCs at pH 4.5 and pH 9.0, respectively, with average γ_{app} values of 0.46 ± 0.17 at pH 4.5 and 0.39 ± 0.13 at pH 9.0.

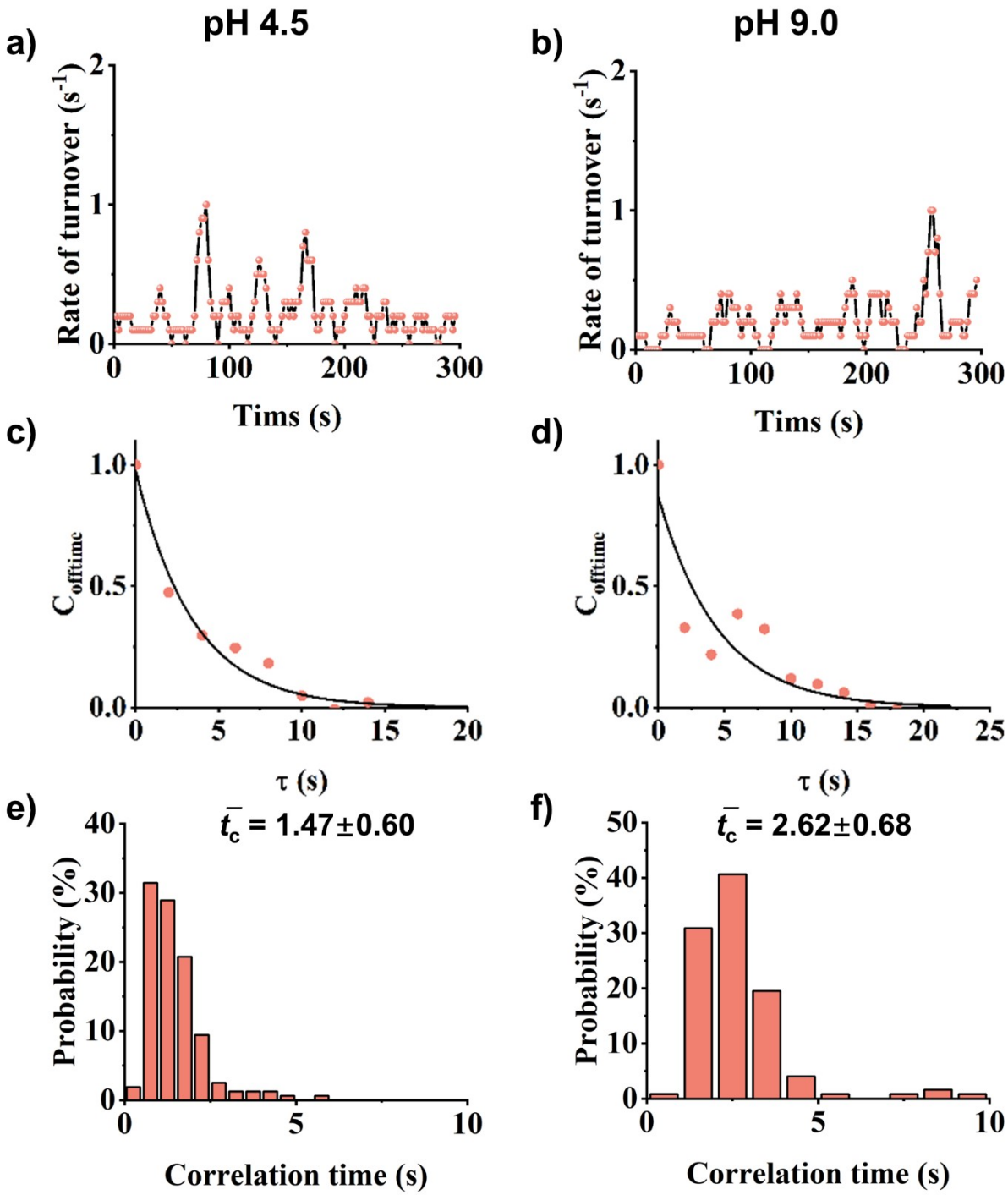

Figure S7. (a-b) Turnover rate of MPA-AuNCs over time at pH 4.5 and pH 9.0, respectively. (c-d) Autocorrelation function C_τ(m) of the microscopic reaction time τ for MPA-AuNCs at pH 4.5 and pH 9.0, respectively. (e-f) Correlation time distribution of MPA-AuNCs at pH 4.5 and pH 9.0, respectively. The average correlation time (t_c) is 1.47 ± 0.60 s at pH 4.5, and 2.62 ± 0.68 s at pH 9.0.

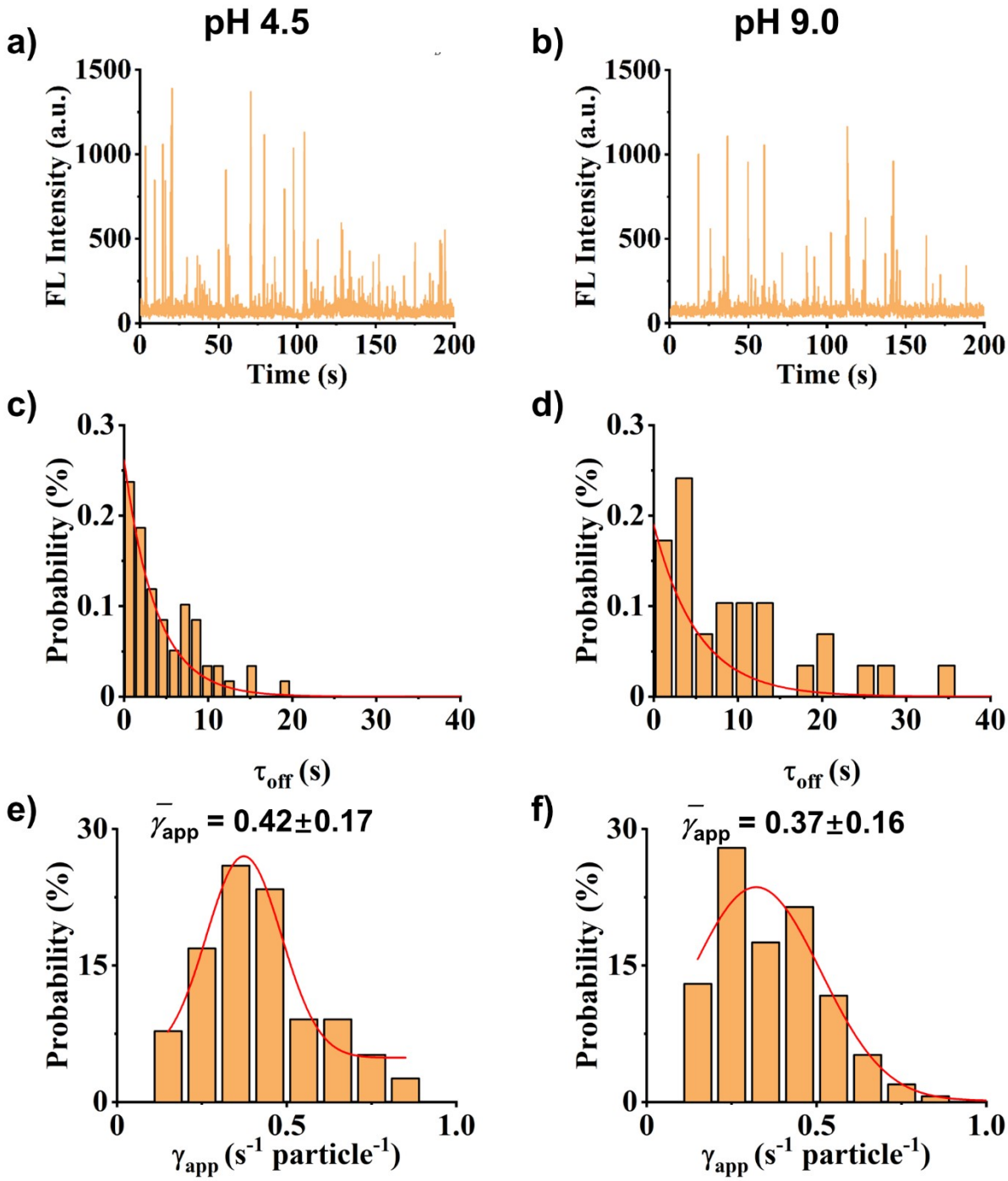

Figure S8. (a-b) Fluorescence intensity-time trajectories of individual GSH-AuNCs at pH 4.5 and pH 9.0, respectively. (c-d) Off-time distribution of GSH-AuNCs at pH 4.5 and pH 9.0, respectively. (e-f) Distribution of γ_{app} for GSH-AuNCs at pH 4.5 and pH 9.0, respectively, with average γ_{app} values of 0.42 ± 0.17 at pH 4.5 and 0.37 ± 0.16 at pH 9.0.

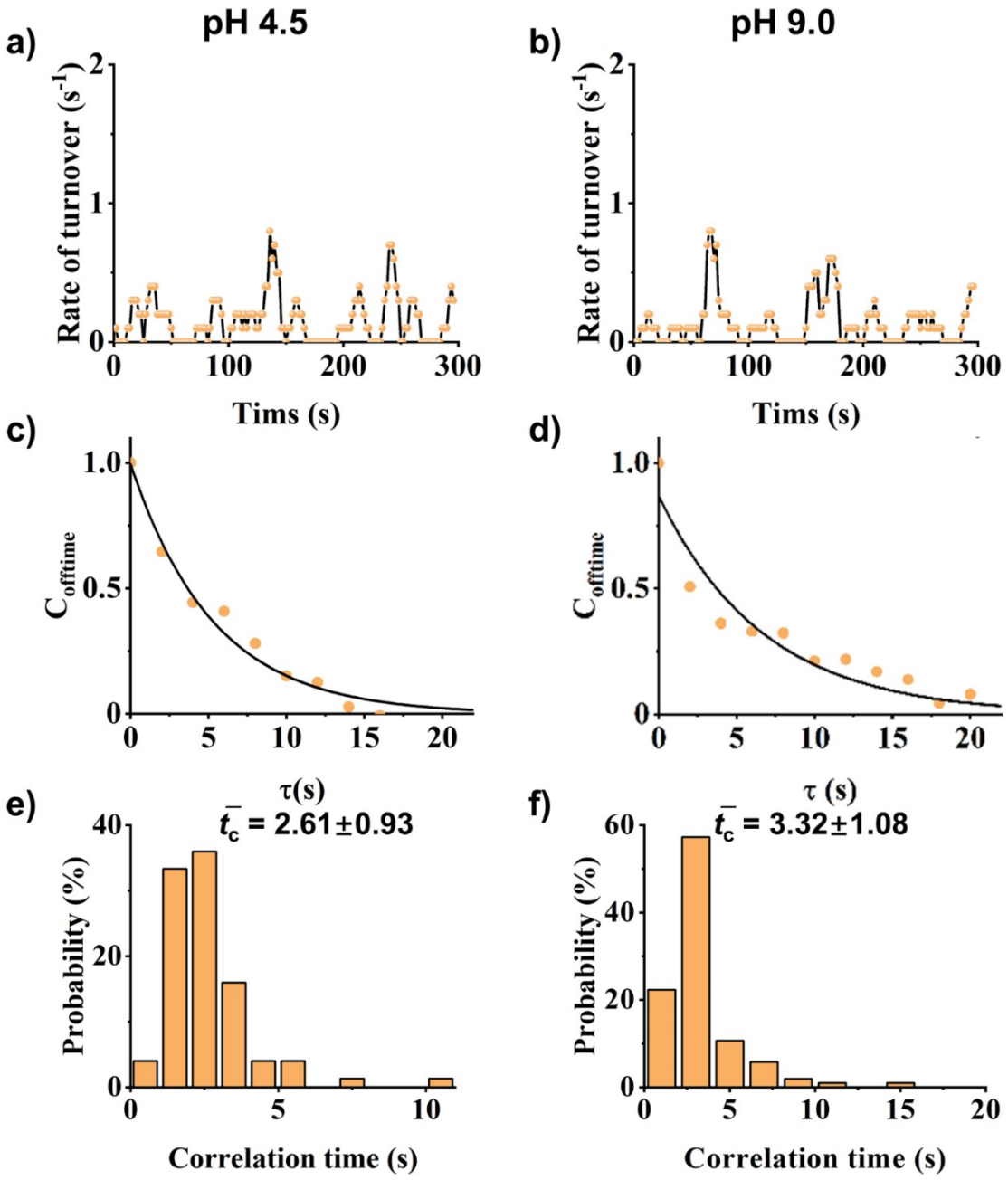

Figure S9. (a-b) Turnover rate of GSH-AuNCs over time at pH 4.5 and pH 9.0, respectively. (c-d) Autocorrelation function $C_\tau(m)$ of the microscopic reaction time τ for GSH-AuNCs at pH 4.5 and pH 9.0, respectively. (e-f) Correlation time distribution of GSH-AuNCs at pH 4.5 and pH 9.0, respectively. The average correlation time is 2.61 ± 0.93 s at pH 4.5, and 3.32 ± 1.08 s at pH 9.0.

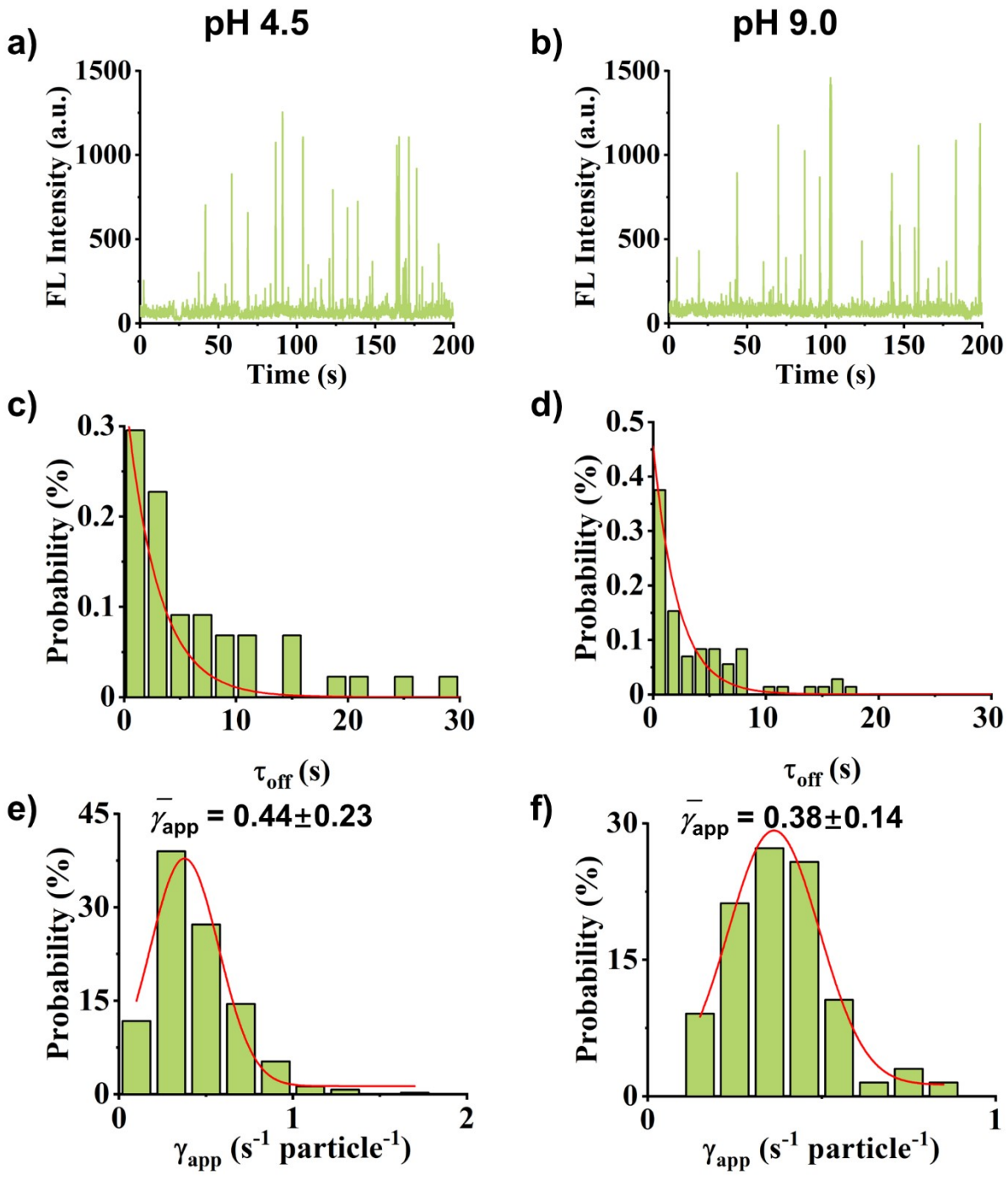

Figure S10. (a-b) Fluorescence intensity-time trajectories of individual KCK-AuNCs at pH 4.5 and pH 9.0, respectively. (c-d) Off-time distribution of KCK-AuNCs at pH 4.5 and pH 9.0, respectively. (e-f) Distribution of γ_{app} for KCK-AuNCs at pH 4.5 and pH 9.0, respectively, with average γ_{app} values of 0.44 ± 0.23 at pH 4.5 and 0.38 ± 0.14 at pH 9.0.

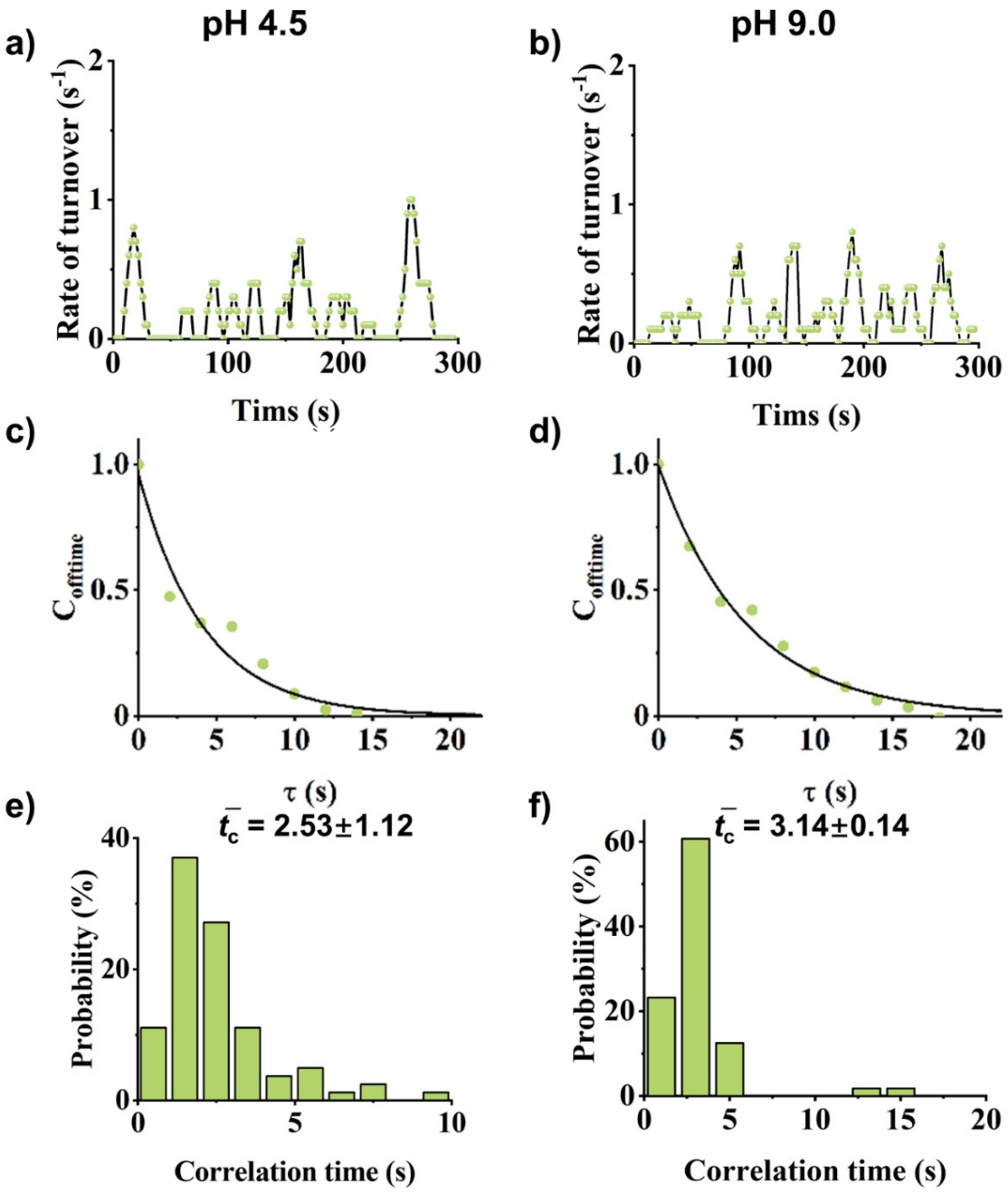

Figure S11. (a-b) Turnover rate of KCK-AuNCs over time at pH 4.5 and pH 9.0, respectively. (c-d) Autocorrelation function $C_\tau(m)$ of the microscopic reaction time τ for GSH-AuNCs at pH 4.5 and pH 9.0, respectively. (e-f) Correlation time distribution of GSH-AuNCs at pH 4.5 and pH 9.0, respectively, with average correlation times $\bar{t}_c$ =2.53 ± 1.12 s at pH 4.5 and t_c =3.14 ± 0.14 s at pH 9.0.

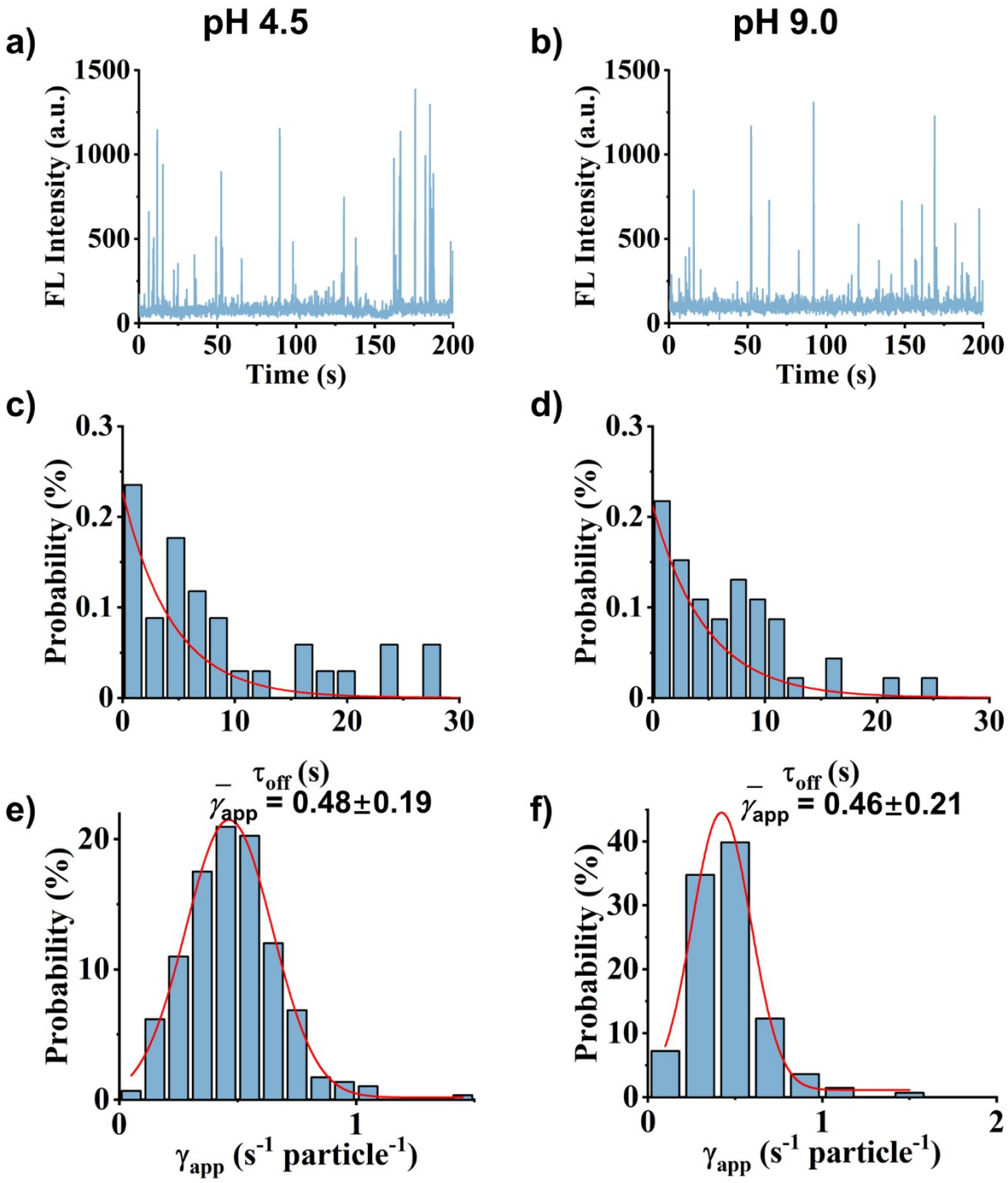

Figure S12. (a-b) Fluorescence intensity-time trajectories of individual BSA-AuNCs particles at pH 4.5 and pH 9.0, respectively. (c-d) Off-time distribution of BSA-AuNCs at pH 4.5 and pH 9.0, respectively. (e-f) Distribution of γ_{app} for BSA-AuNCs at pH 4.5 and pH 9.0, respectively, with average γ_{app} values of 0.48 ± 0.19 at pH 4.5 and 0.46 ± 0.21 at pH 9.0.

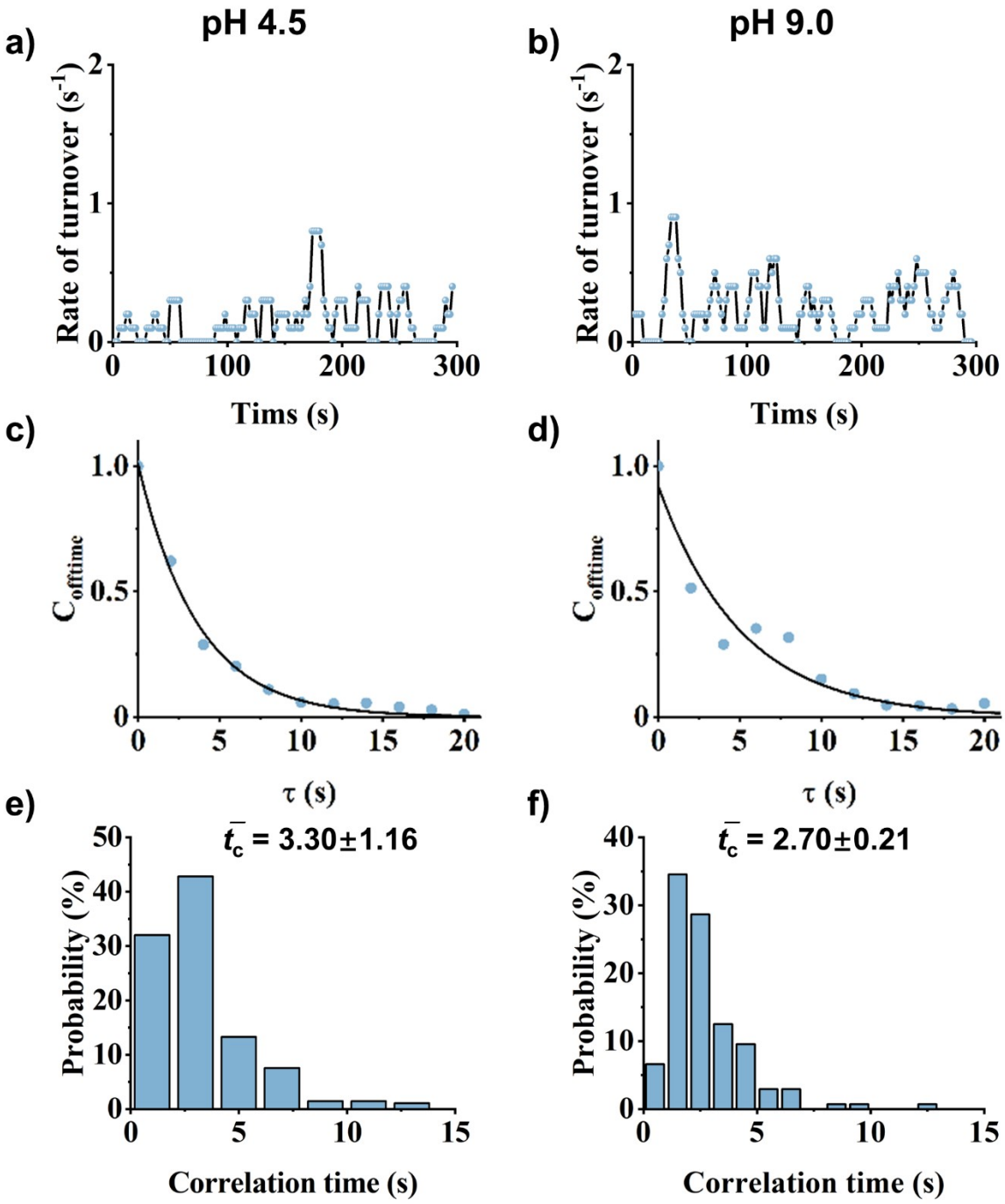

Figure S13. (a-b) Turnover rate of BSA-AuNCs over time at pH 4.5 and pH 9.0, respectively. (c-d) Autocorrelation function C_τ(m) of the microscopic reaction time τ for BSA-AuNCs at pH 4.5 and pH 9.0, respectively. (e-f) Correlation time distribution of BSA-AuNCs at pH 4.5 and pH 9.0, respectively, with average correlation times t_c = 3.30 ± 1.16 s at pH 4.5 and t_c = 2.70 ± 0.21 s at pH 9.0.

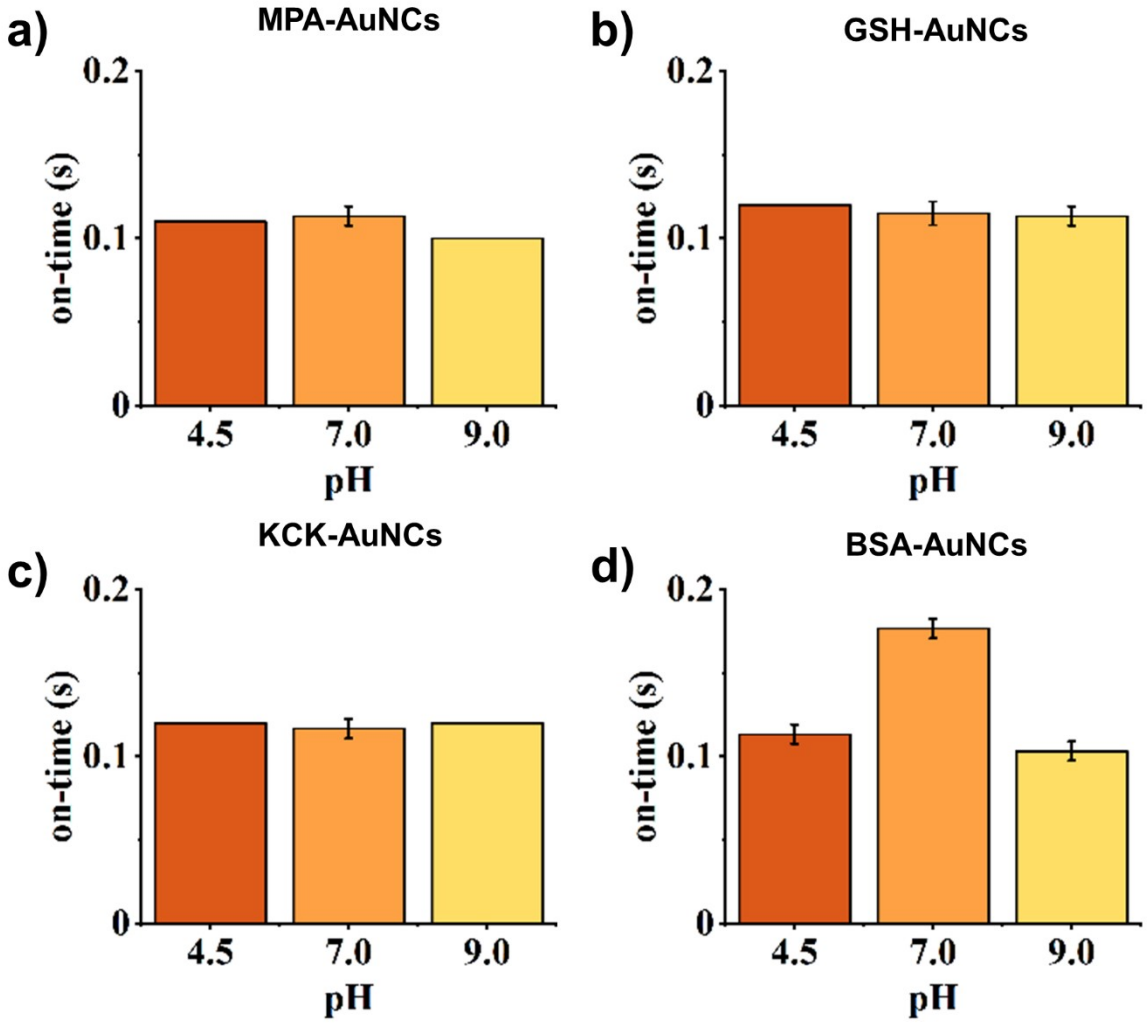

Figure S14. Changes in on-time during the single-molecule catalytic process for the four AuNCs at different pH values.

REFERENCES

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2. M. Wang, Z. Wu, J. Yang, G. Wang, H. Wang and W. Cai, $Au_{25}(SG)_{18}$ as a fluorescent iodide sensor, *Nanoscale.*, 2012, **4**, 4087-4090; Y. Shichibu, Y. Negishi, H. Tsunoyama, M. Kanehara, T. Teranishi and T. Tsukuda, Extremely high stability of glutathionate-protected Au_{25} clusters against core etching, *Small.*, 2007, **3**, 835-839.
3. J. Xie, Y. Zheng and J. Y. Ying, Protein-Directed Synthesis of Highly Fluorescent Gold Nanoclusters, *J. Am. Chem. Soc.*, 2009, **131**, 888-889.