Supporting Information

DNA Origami-Templated Individual Gold Nanocluster: Probing The Photophysical Dynamics Using Single Molecule Fluorescence Spectroscopy

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Table S1. Poly-C strand sequences

Poly-c	5-TTTCATTTGGTCAATAACCTGTTTATATCGCGCCCCCCCCCCCCCCCCCCCC-3’

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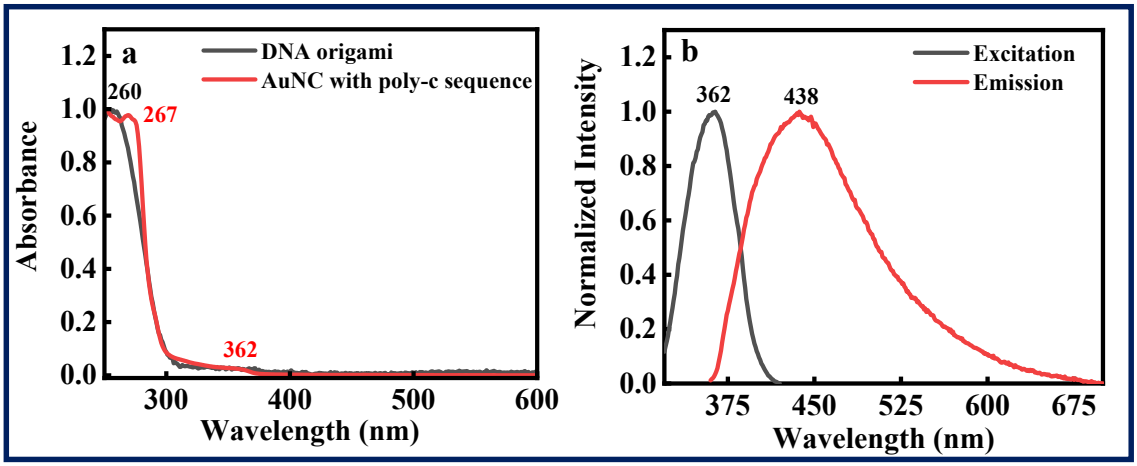


Figure S1. (a) UV-Vis spectra of the DNA origami and Au NC with poly-C sequence, (b) Fluorescence spectra of the as-prepared Au NCs with poly-C sequence.

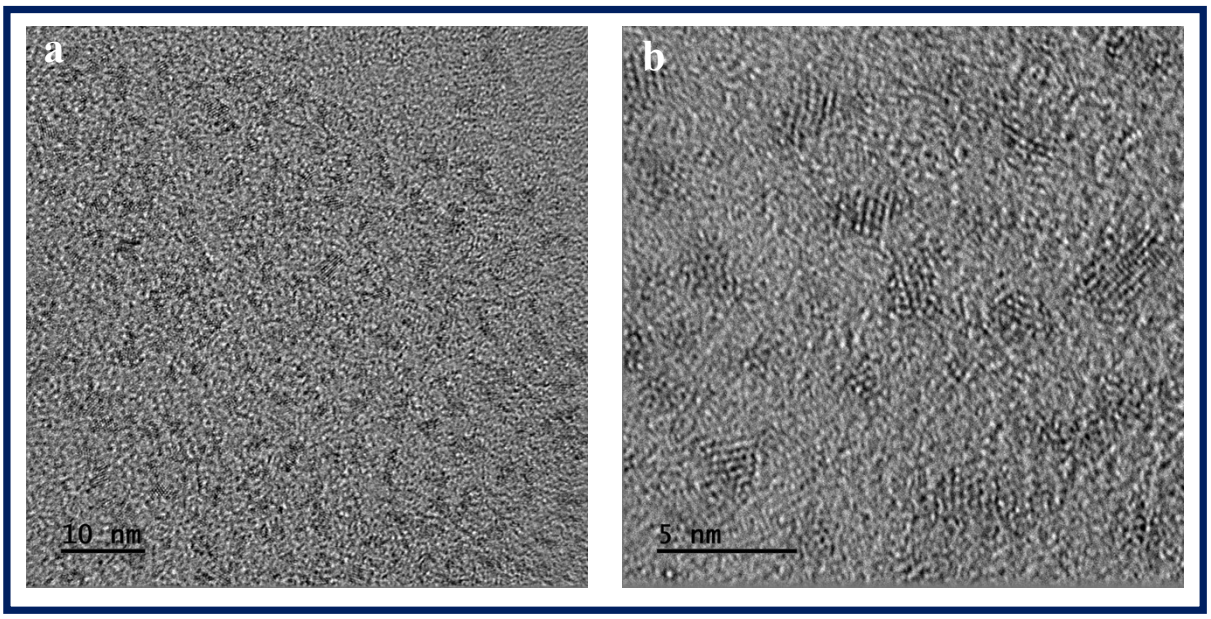


Figure S2. (a) TEM images of Au NCs synthesized by poly-C sequence, and (b) HRTEM images.

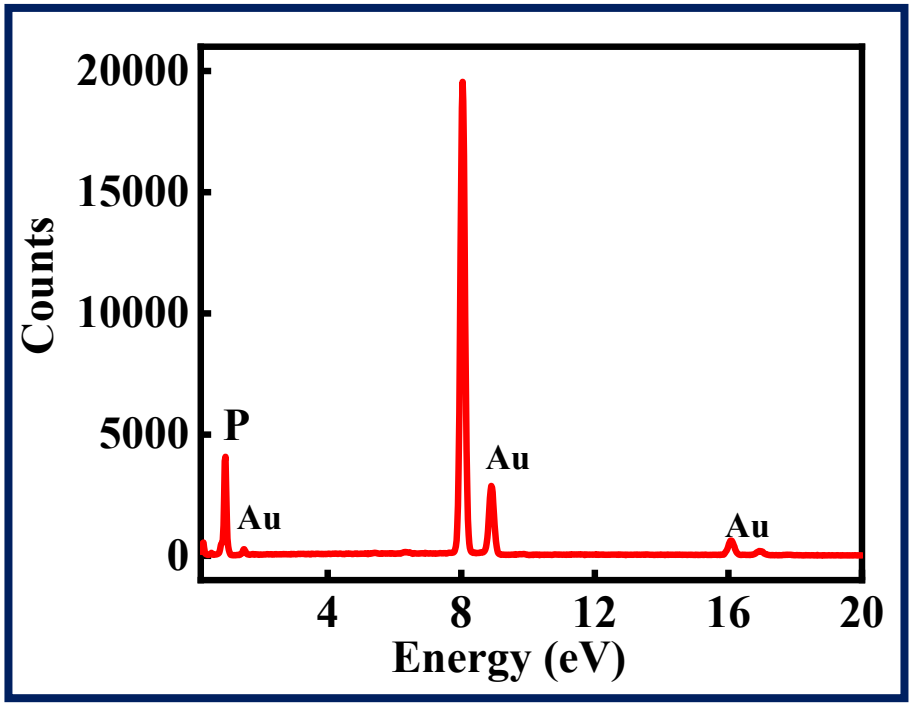


Figure S3. EDX spectrum of Au NC synthesized by poly-C sequence.[1]

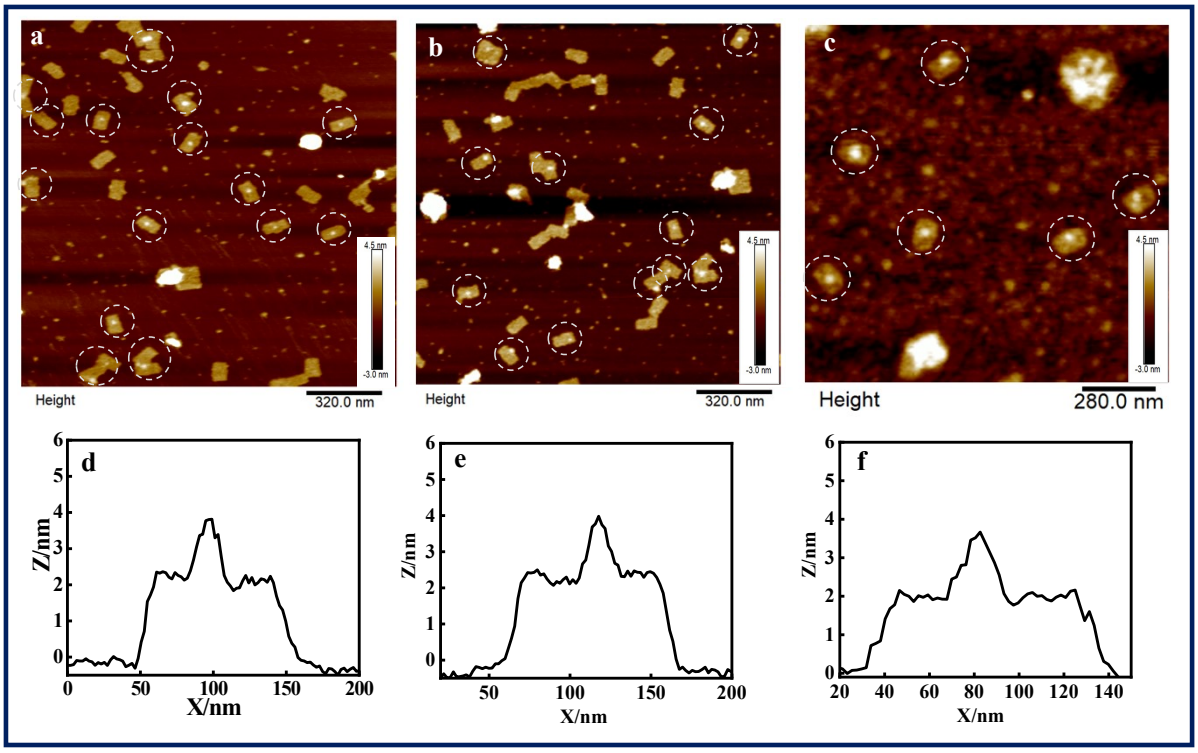


Figure S4. (a,b and c) AFM images of Au NC on DNA origami, and (d,e, and f) corresponding AFM height profiles.

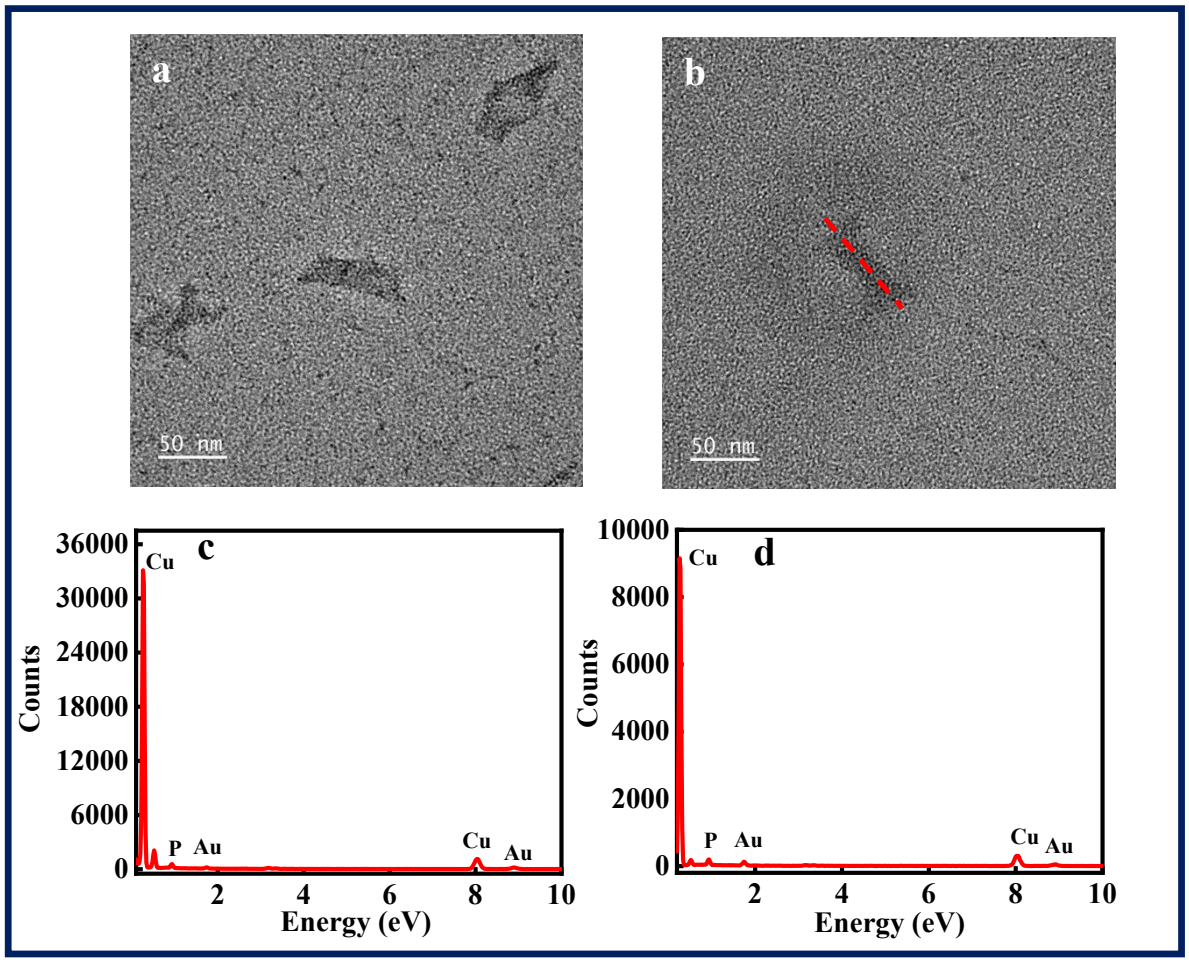


Figure S5. (a and b) TEM images of Au NC on DNA origami monomer, (c) EDX spectrum, and (d) Line scan EDX.

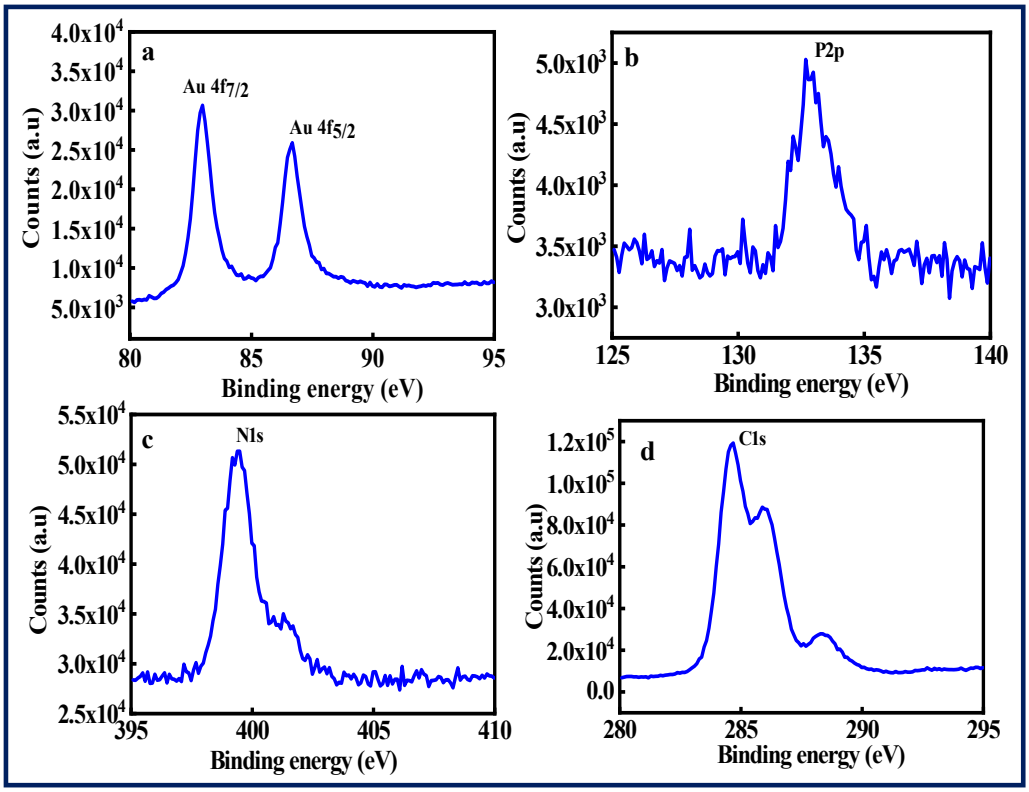


Figure S6. XPS spectrum of Au NC on DNA origami.[2]

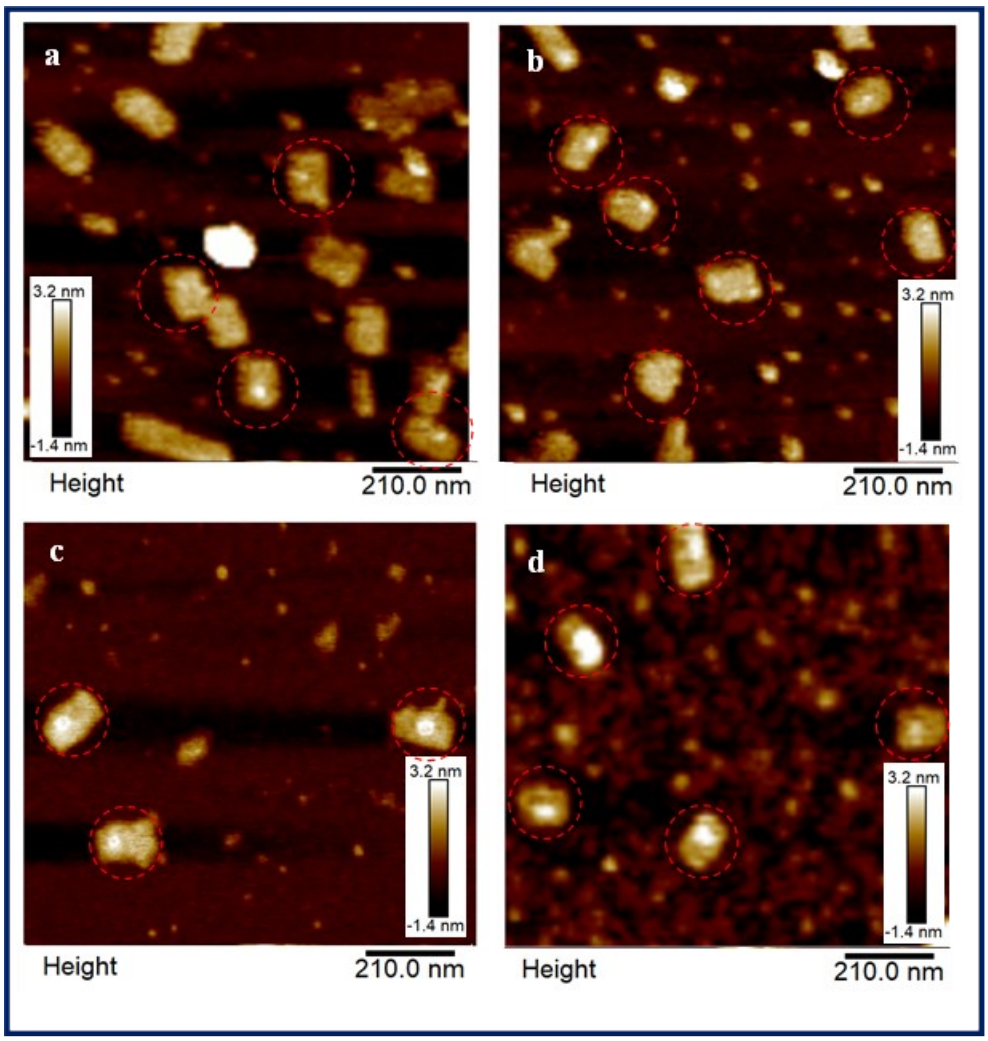


Figure S7. AFM images of Au NC on DNA origami (a and b) with different Au concentrations of 1 μM and 4 μM, respectively, and (c and d) with different temperatures of 35 ^{0}C and 45 ^{0}C, respectively.

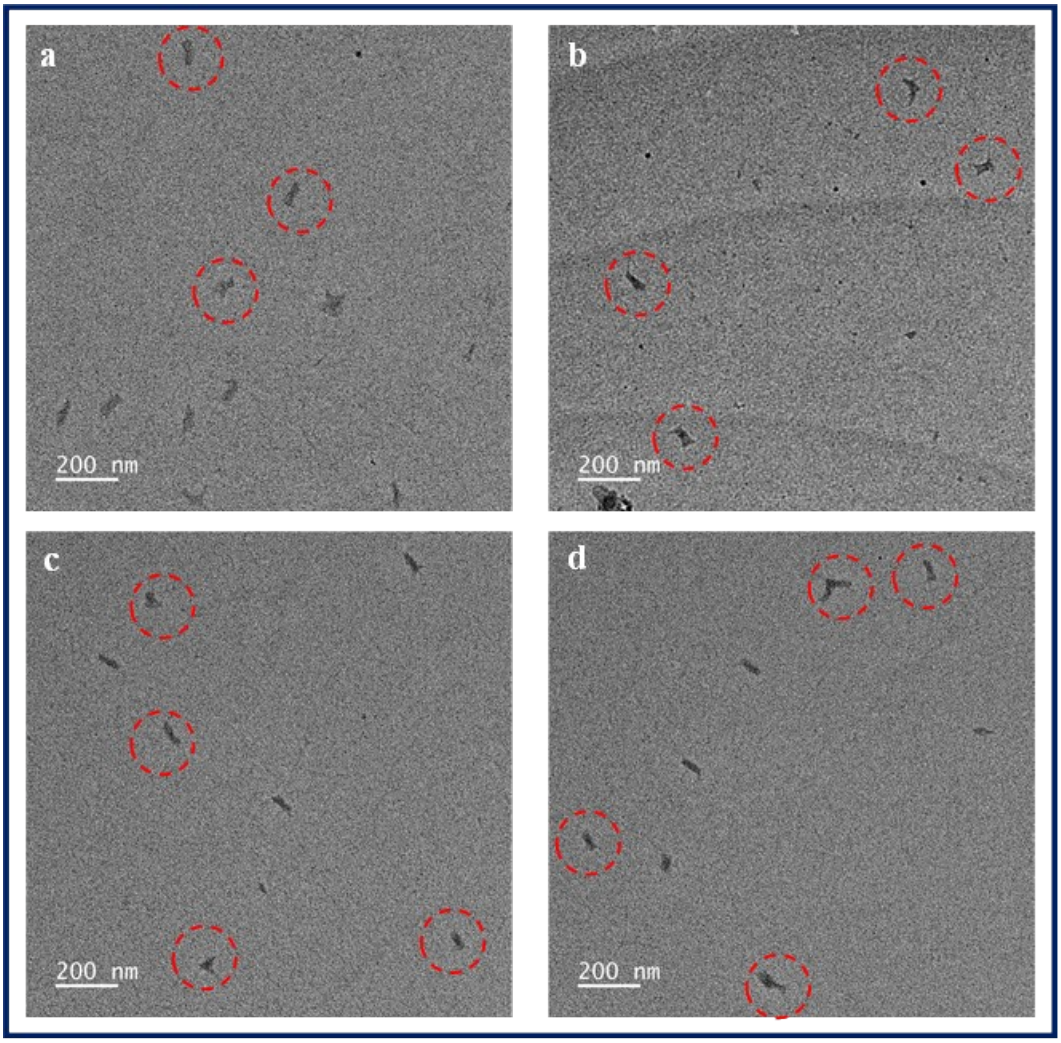
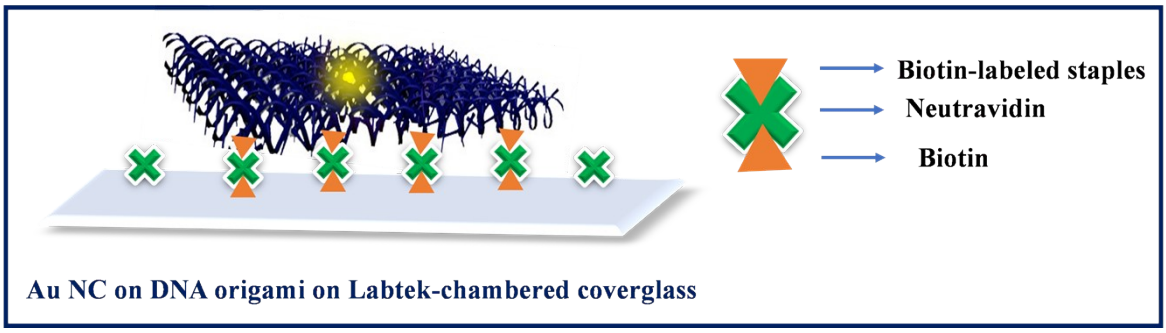


Figure S8. TEM images of Au NC on DNA origami (a and b) with different Au concentrations of 1 µM and 4 µM, respectively, and (c and d) with different temperatures of 35 ^{0}C and 45 ^{0}C, respectively.

Table S2. Biotin modified staple strand sequences on DNA origami.

Name	Sequence 5’ to 3’
Bio-11	Biotin- TTGAGAATAGCTTTTGCGGGATCGTCGGGTAGCA
Bio-27	Biotin-TTGGAAAGCGACCAGGCGGATAAGTGAATAGGTG
Bio-188	Biotin- TTTGGTTTTTAACGTCAAAGGGCGAAGAACCATC
Bio-205	Biotin- GCCAACAGTCACCTTGCTGAACCTGTTGGCAA



Scheme S1. Immobilization of an Au NC on DNA origami on a Labtek-Chambered coverglass using Biotin-Neutravidin linkage.

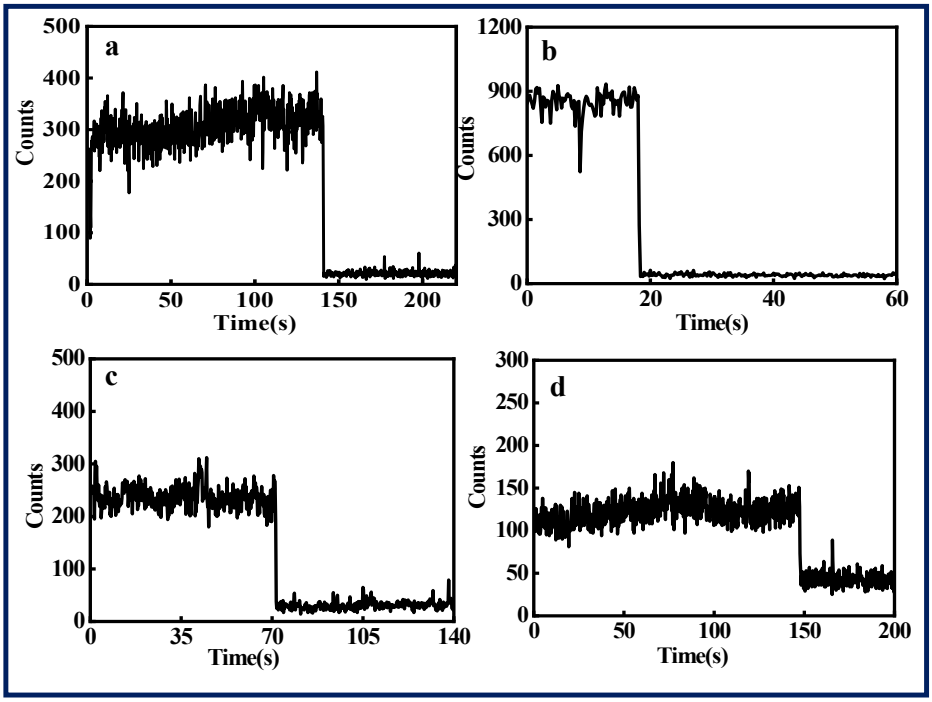


Figure S9. Time trace profiles for single-step photobleaching of Au NC on DNA origami.

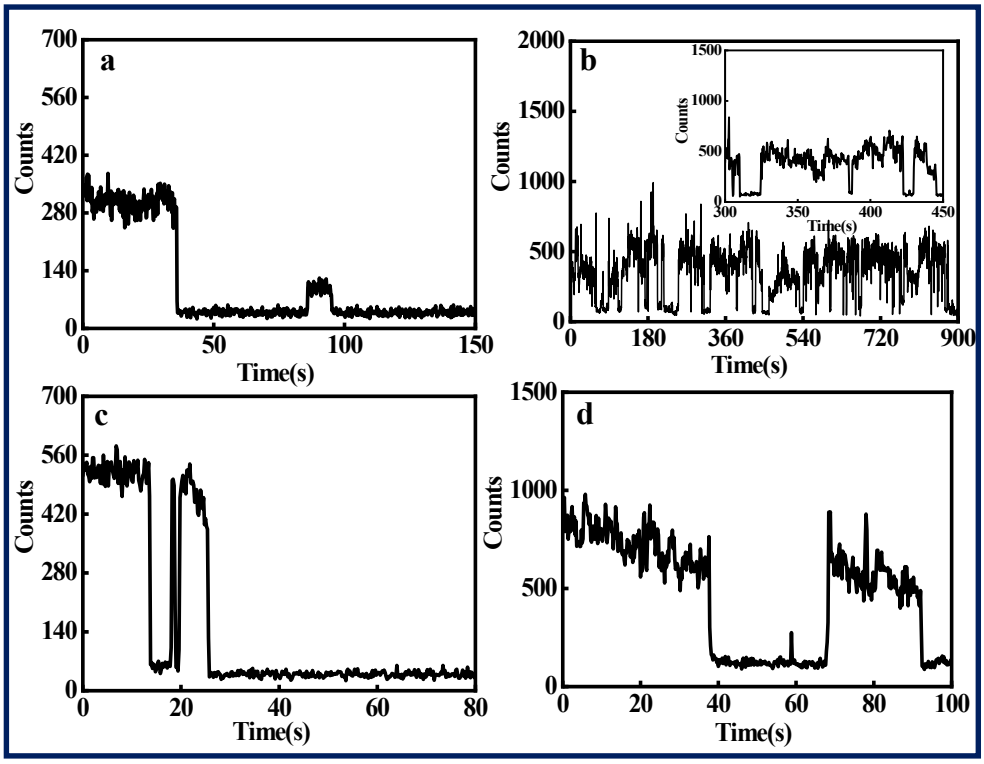


Figure S10. Time trace profiles showing blinking of Au NC on DNA origami.

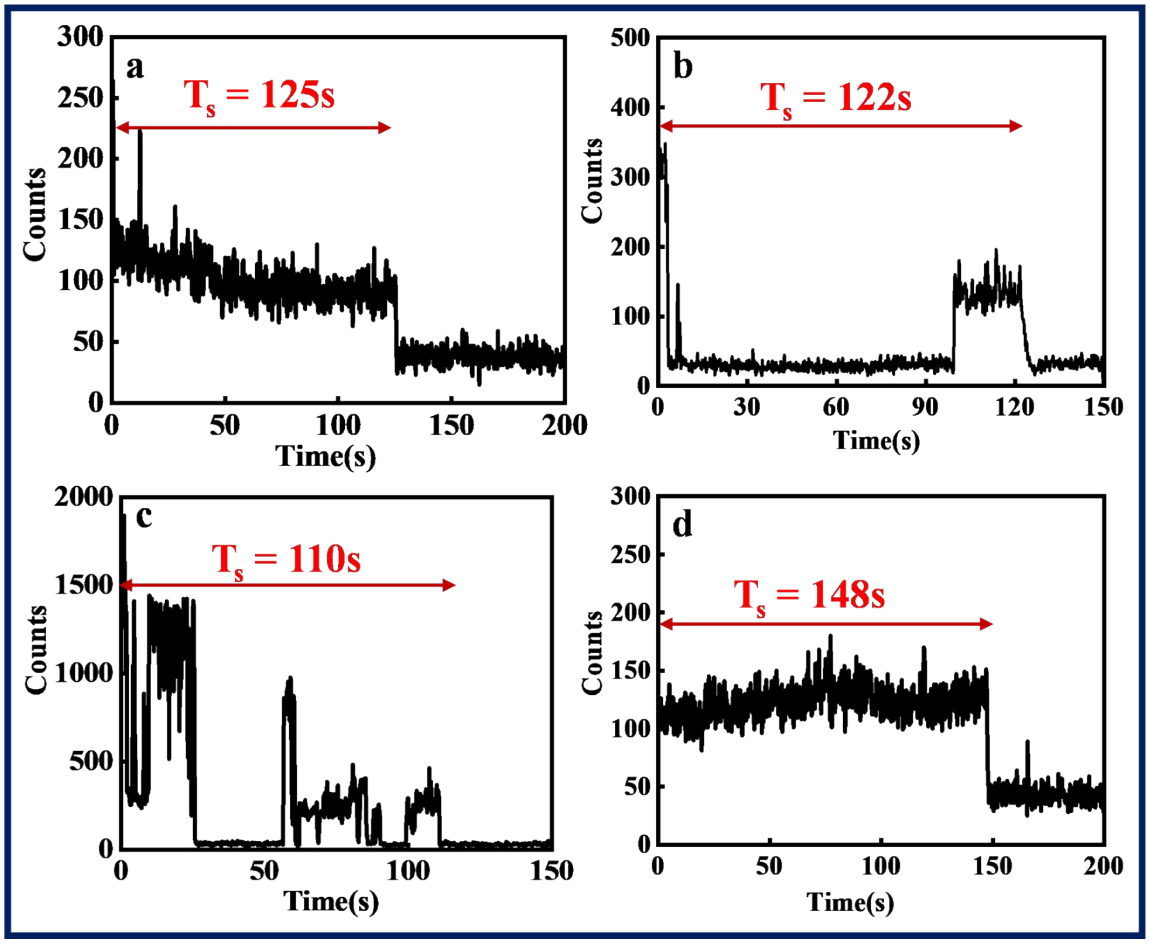


Figure S11. Fluorescence time traces of Au NC on DNA origami indicating their survival times.

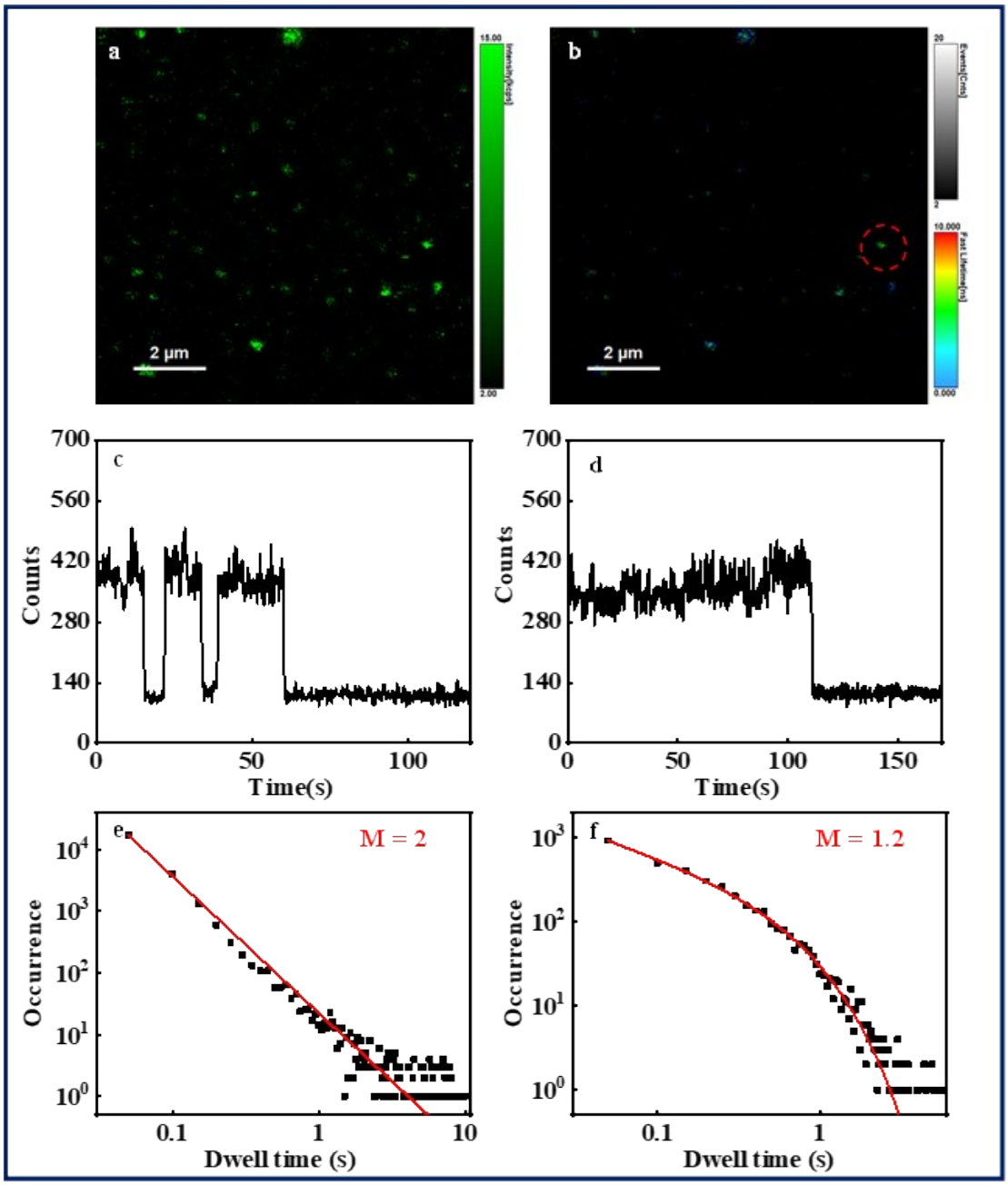


Figure S12. Single-molecule confocal fluorescence measurements with 0.035 μW laser intensity (a) fluorescence intensity images, (b) FLIM images, (c and d) fluorescence transient profiles, and statistical distribution for (e) on-events, and (f) off-events of Au NC on DNA origami.

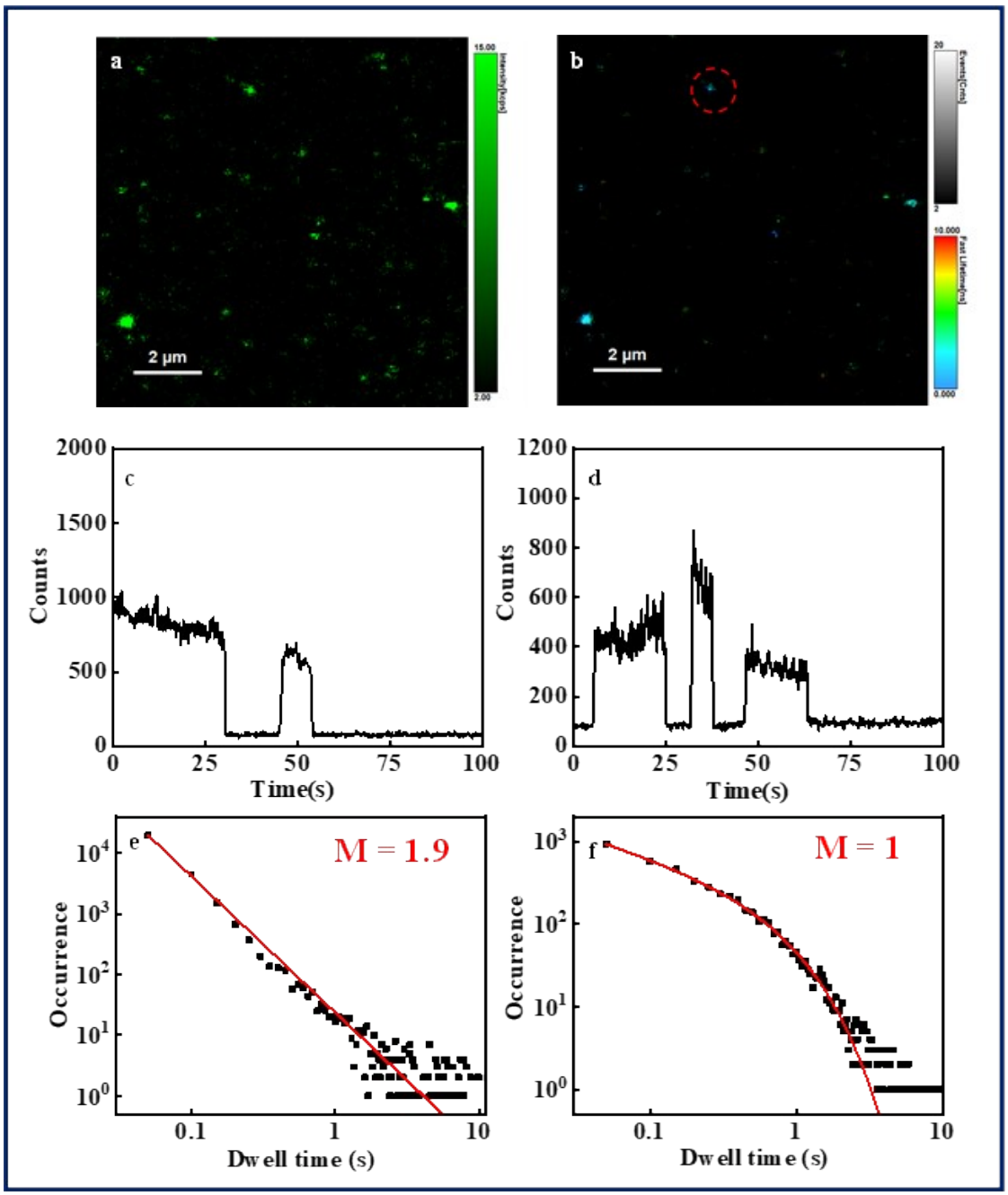


Figure S13. Single-molecule confocal fluorescence measurements with 0.14 μW laser intensity (a) fluorescence intensity images, (b) FLIM images, (c and d) fluorescence transient profiles, and statistical distribution for (e) on-events, and (f) off-events of Au NC on DNA origami.

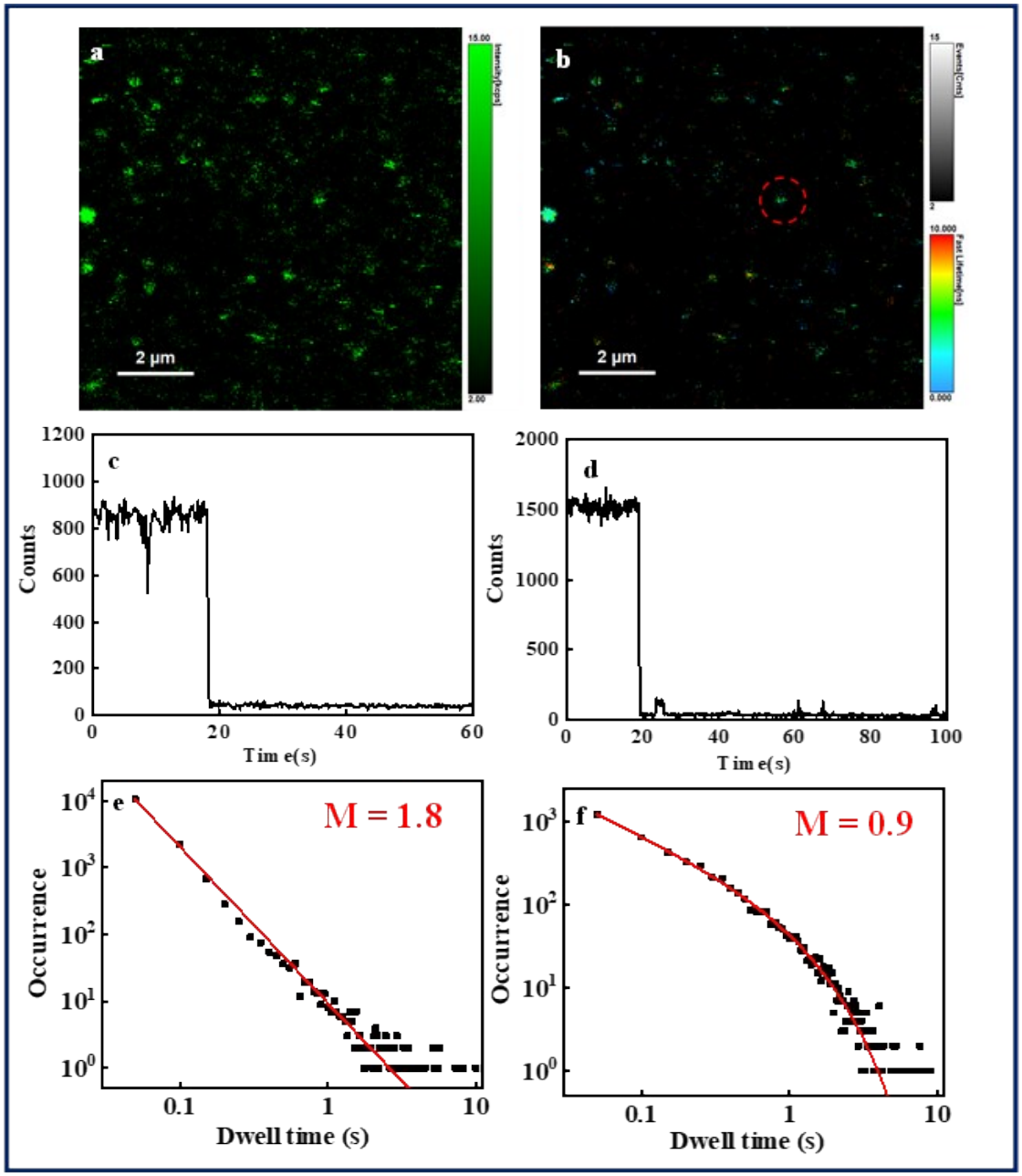


Figure S14. Single-molecule confocal fluorescence measurements with 0.28 μW laser intensity (a) fluorescence intensity images, (b) FLIM images, (c and d) fluorescence transient, and statistical distribution for (e) on-events, and (f) off-events of Au NC on DNA origami.

Table S3. The comparative analysis of the survival times of the clusters with our designed Au NC on DNA origami and previously reported NCs.

Cluster	**Survival time**	**Reference**
Ag NC	65 s	[3]
AuAg NC	218 s	[3]
Au NC	18 s	[2]
Ag NC	15 s	[4]
DNA-Hosted Silver Nanoclusters	360 s	[5]
Au NC	5 s	[6]
Au NC synthesized with poly-C sequences	85 s	This work
Au NC on DNA origami	880 s	This work

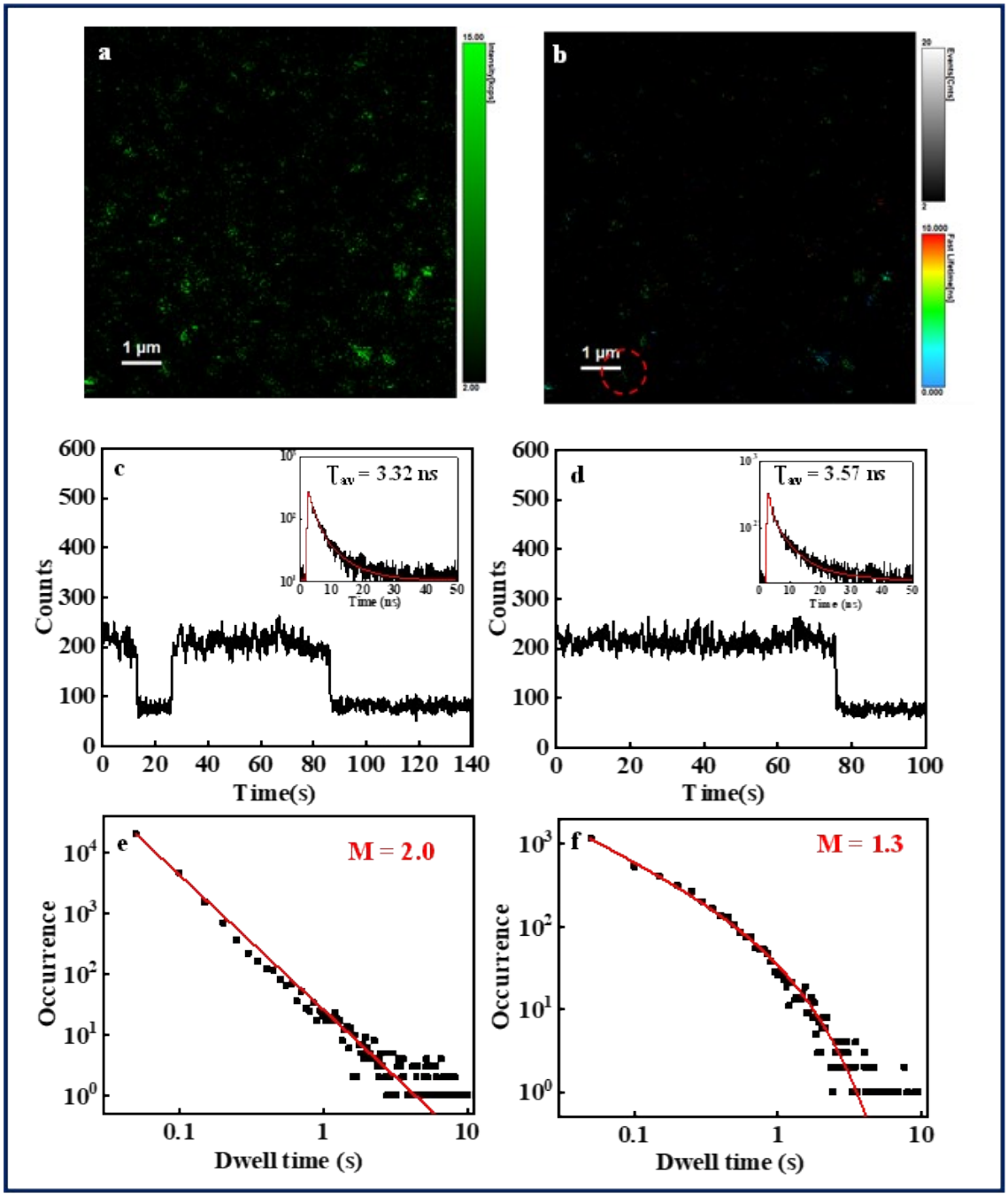


Figure S15. Single-molecule confocal fluorescence measurements (a) fluorescence intensity images, (b) FLIM images, (c and d) fluorescence transient profiles (inset graph shows fluorescence decay time (τ_{av} value) of Au NCs synthesized by poly-C sequences), and statistical distribution for (e) on-events, and (f) off-events of Au NCs synthesized by poly-C sequences.

References:

1. S. Chakraborty, S. Babanova, R. C. Rocha, A. Desireddy, K. Artyushkova, A. E. Boncella, P. Atanassov and J. S. Martinez, *J. Am. Chem. Soc.*,2015, **137**, 11678-11687.
2. A. Yadav, N. C. Verma, C. Rao, P. M. Mishra, A. Jaiswal and C. K. Nandi, *J. Phys. Chem. Lett.*, 2020, **11**, 5741-5748.
3. Rashi, V. Kaur, A. Devi, D. Bain, T. Sen and A. Patra, *J. Phys. Chem. Lett.*, 2023, **14**, 10166-10172.
4. A. Yadav, K. Kaushik, S. Sharma, F. Anjum and C. K. Nandi, *ACS Appl. Nano Mater.*, 2022, **5**, 9260-9265.
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