Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Microfluidic-assisted Biomineralization of CRISPR/Cas9 in Near-infrared Responsive Metal-organic Frameworks for Programmable Gene-editing

Xiaoyu Xu, ‡[a,b,c] Chang Liu, ‡[b] Shengyi Wang, ‡[a,b,c] , Ermei Mäkilä,[d] Jiali Wang,[a,c] Oliver Koivisto,[b] Junnian Zhou,[b,e] Jessica M. Rosenholm,[b] Yilai Shu[a,c] and Hongbo Zhang*[b,f,g]*

[a.] ENT Institute and Department of Otorhinolaryngology, Eye & ENT Hospital, State Key Laboratory of Medical Neurobiology, Institutes of Biomedical Sciences, Fudan University, Shanghai 200031, China
[b.] Pharmaceutical Sciences Laboratory, Faculty of Science and Engineering, Åbo Akademi University, Turku 20520, Finland
[c.] NHC Key Laboratory of Hearing Medicine (Fudan University), Shanghai, 200031, China
[d.] Laboratory of Industrial Physics, Department of Physics and Astronomy, University of Turku, Turku 20500, Finland
[e.] Stem Cell and Regenerative Medicine Lab, Beijing Institute of Radiation Medicine, Beijing 100850, China
[f.] Turku Bioscience Centre, University of Turku and Åbo Akademi University, Turku 20520, Finland
[g.] The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China
* Corresponding authors: hongbo.zhang@abo.fi (H. Zhang), yilai_shu@fudan.edu.cn (Y. Shu)
‡ These authors contributed equally to this study.

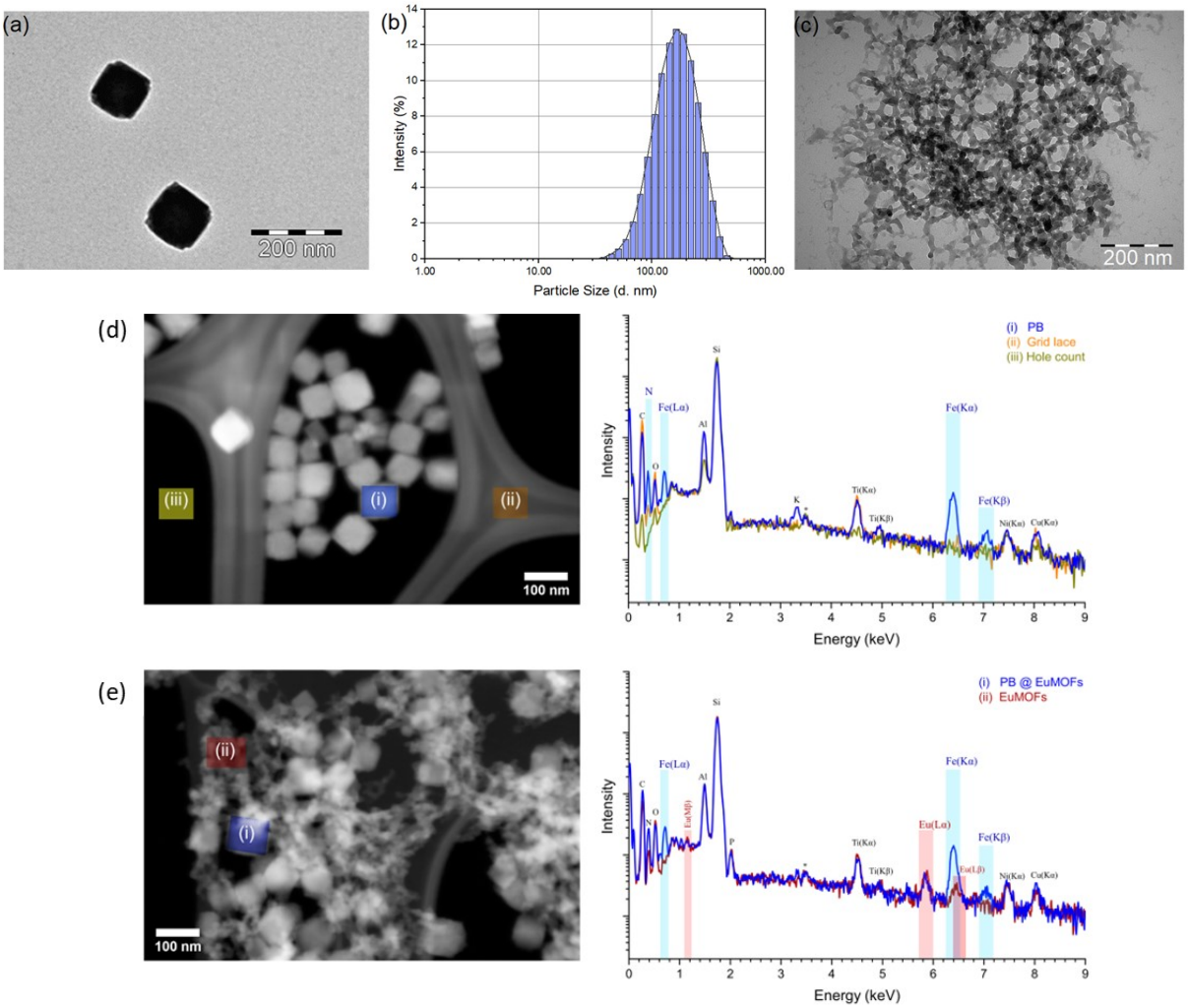


Figure S1. TEM image (a) and particle size distribution (b) at room temperature measured by DLS of PB, (c) TEM image of prepared PB@EuMOFs. High-angle annular darkfield (HAADF) STEM images and EDS spectra of highlighted positions from PB particles (d) and PB@EuMOFs (e).

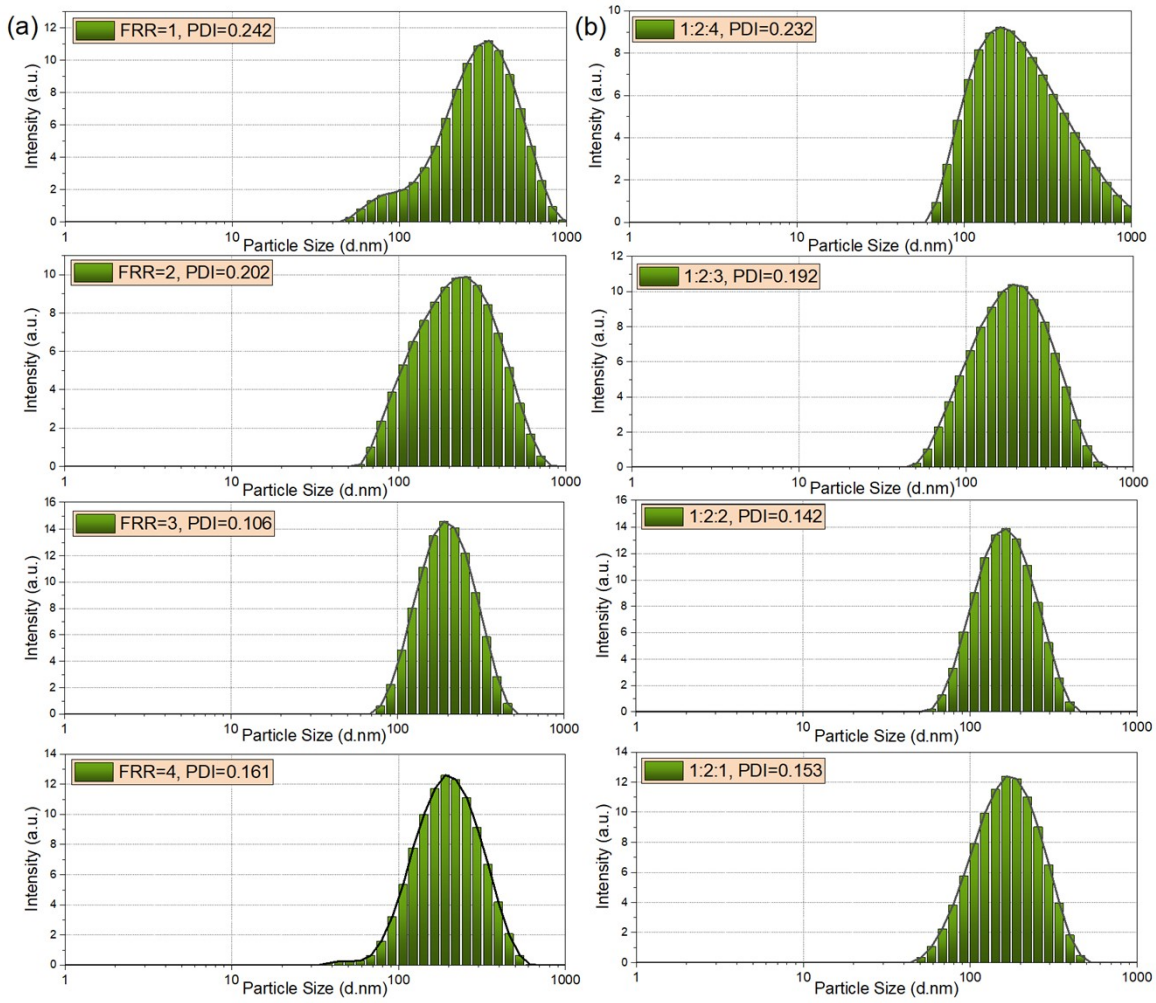


Figure S2. The particle size distribution of microfluidic-prepared PB@EuMOFs (0.5 mg/mL) in different FRR (a) and concentration ratio of reactants (b) measured by DLS.

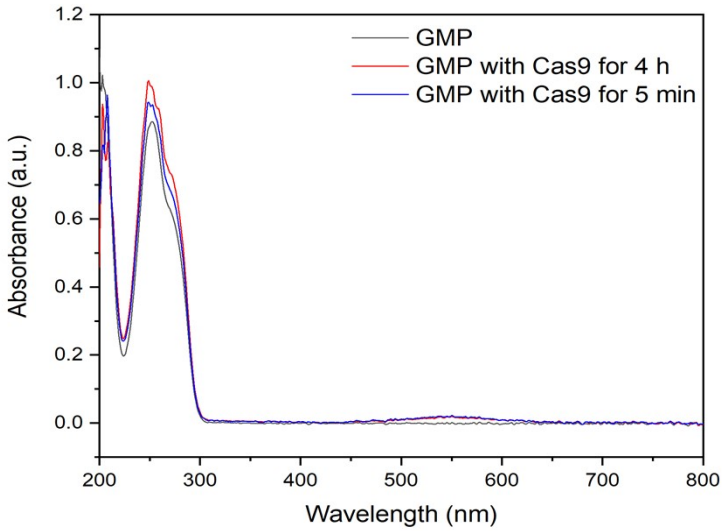


Figure S3. UV-vis absorption spectra of pure GMP solution (2 mM) and GMP with Cas9 protein (40 ng/μL) at timepoints of 5 min and 4 h.

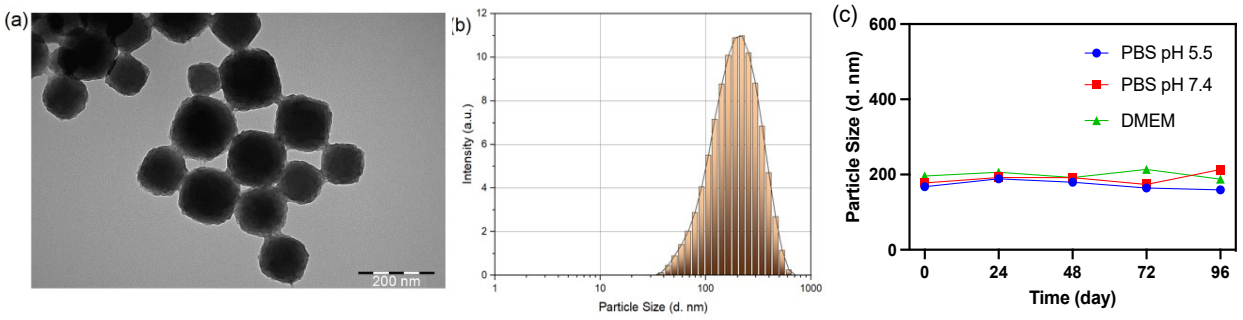


Figure S4. TEM image (a), size distribution, d_{nm}=165.7, PDI=0.205 (b) and solution stability within 96 h (c) measured by DLS of PB@RNP-EuMOFs from microfluidic method.

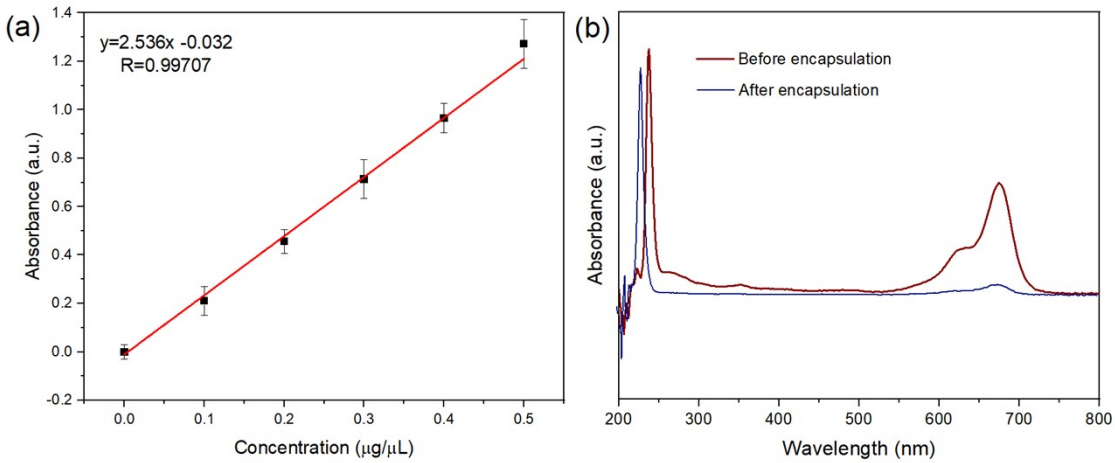


Figure S5. (a) Calibration curve of Cy5.5 labeled Cas9 determined by UV-vis spectrophotometric (λ_{max}=678 nm); (b) UV-vis absorption spectroscopy of Cy5.5 labeled Cas9 before and after encapsulation.

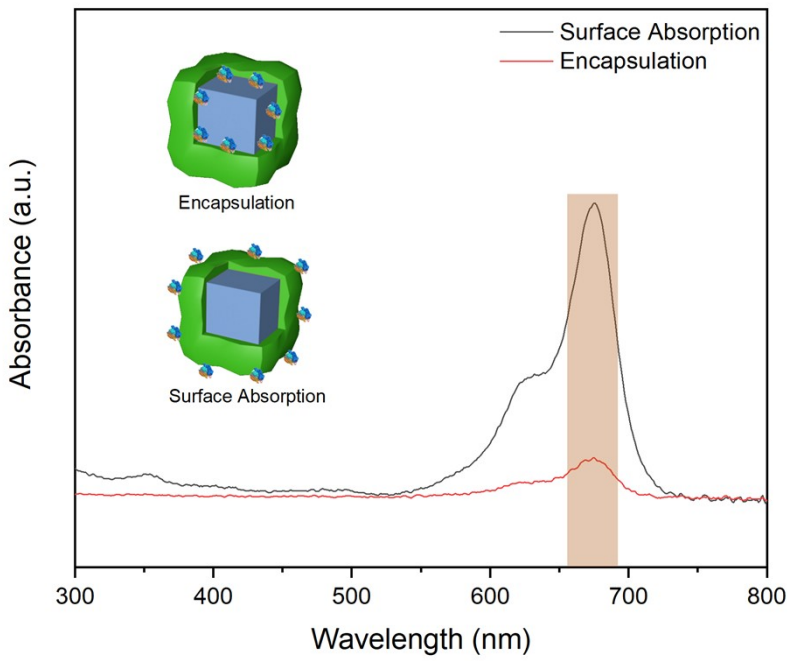


Figure S6. PVP-surface-adsorbent exchange experiment for encapsulated and surface adsorbed RNP in PB@EuMOFs. The inserts were picture illustration of different loading patterns.

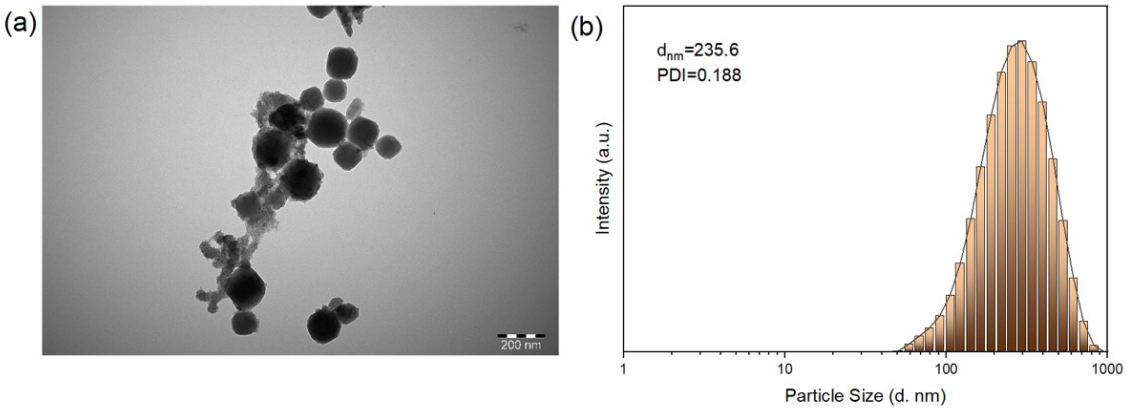


Figure S7. TEM image (a) and size distribution measured by DLS (b) of PB@RNP-EuMOFs from bulk method. d_{nm}=235.6, PDI=0.213.

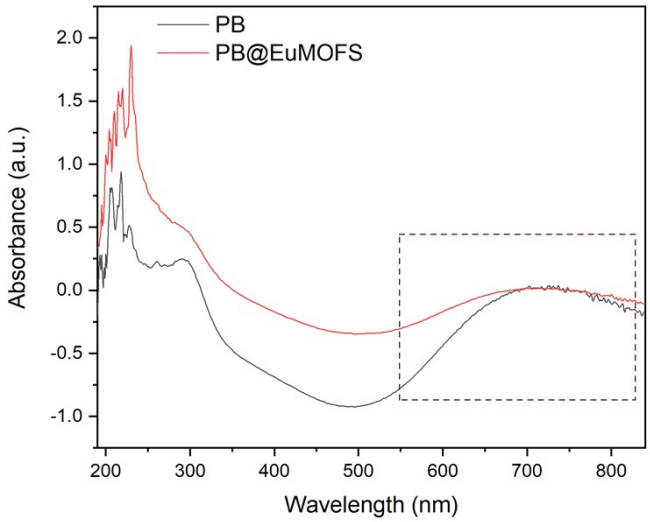


Figure S8. UV-vis absorption spectroscopy of PB and PB@EuMOFs in water.

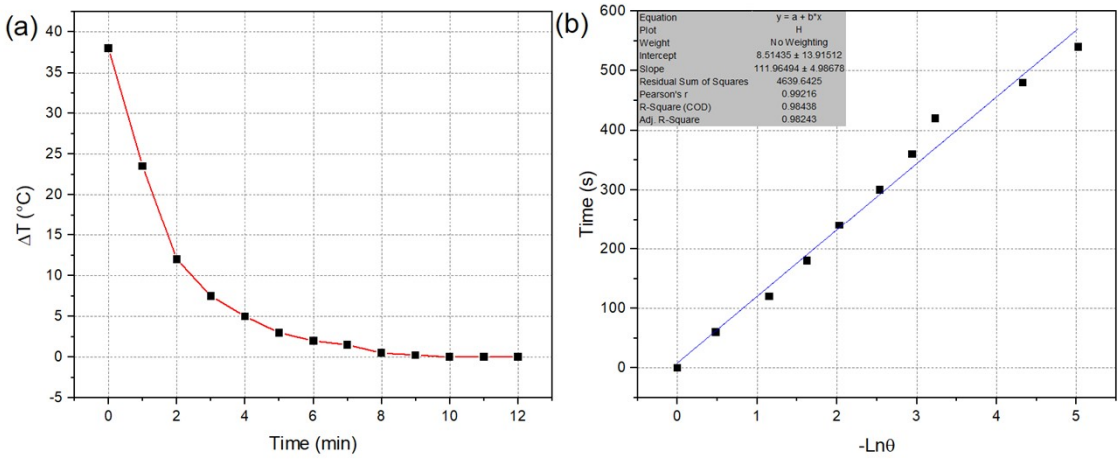


Figure S9 (a) The cooling curve of PB@EuMOFs aqueous solution (50 μg/mL) for 12 min after laser shutting off (808 nm, 2 W/cm^2); (b) Linear time data versus -lnθ obtained from the cooling period of Figure S9a.

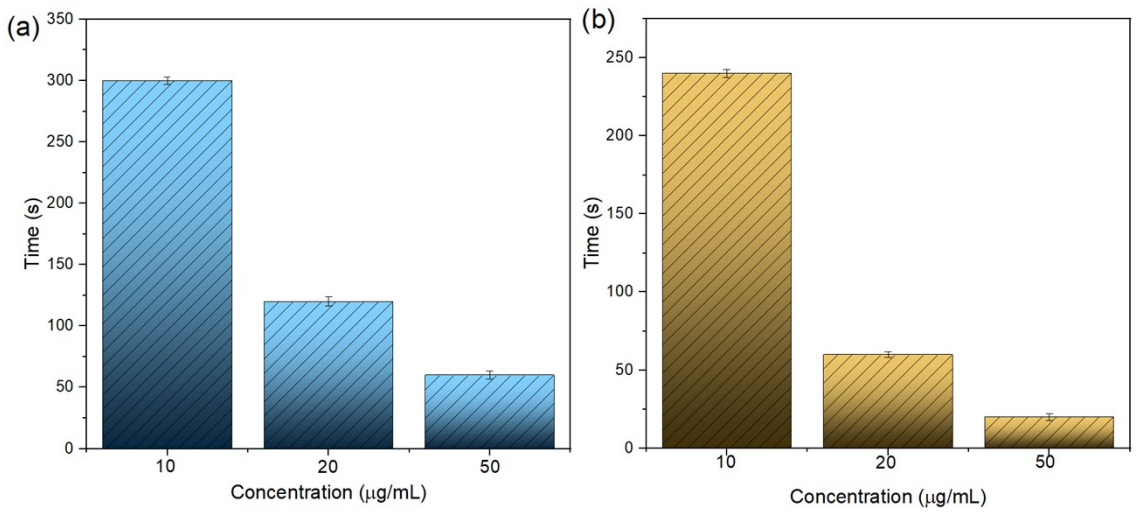


Figure S10. The time taking of PB@EuMOFs in different concentration (10, 20, 50 μg/mL) from 25 ℃ (a) and 37 ℃ (b) to 42 ℃ under continuous irradiation. The infrared thermal camera was used to monitoring the temperature change.

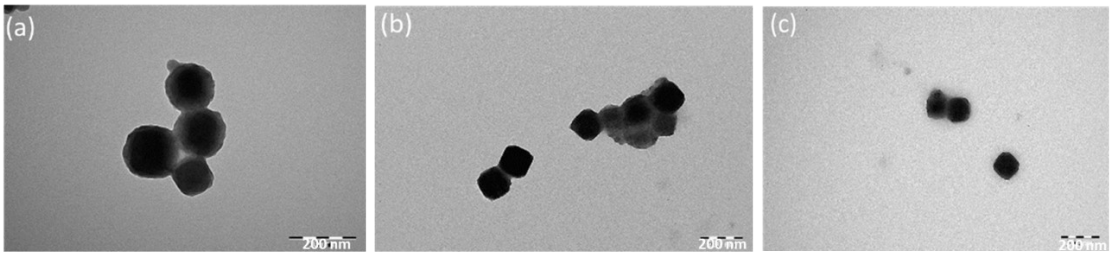


Figure S11. Mophology of PB@EuMOFs after different temperature treatment: (a) 25 ℃, (b) 37 ℃ and (c) 42 ℃ detected by TEM.

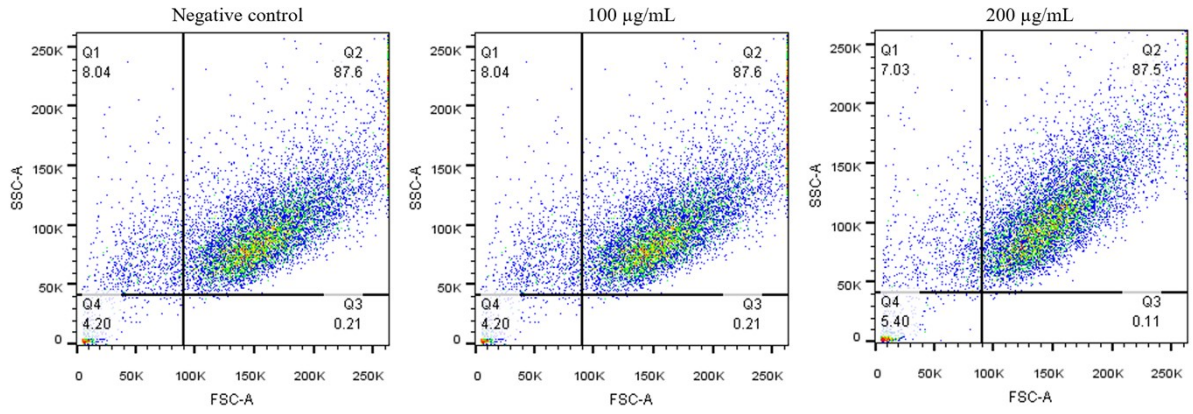


Figure S12. Cell viability analysis of PB@EuMOFs in Hela/GFP cells after laser irritation (three times) determined by flow cytometry. The cells were collected after 48 h incubation.

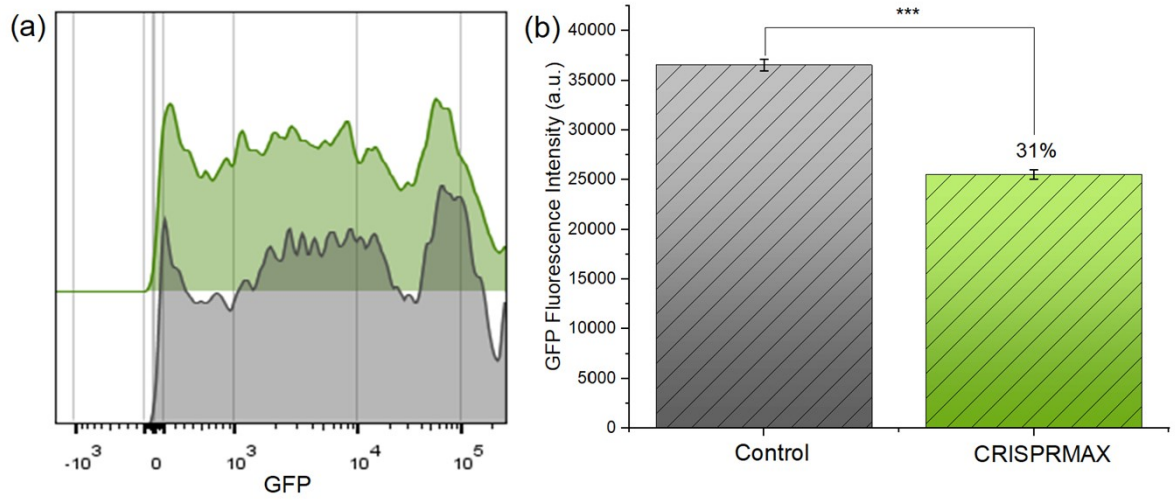


Figure S13. GFP gene editing efficiency of Lipfectamine CRISPRMAX in Hela/GFP cells detected by flow cytometry (a) and GFP fluorescence intensity (b). Bars represent mean ± SD (n=3).

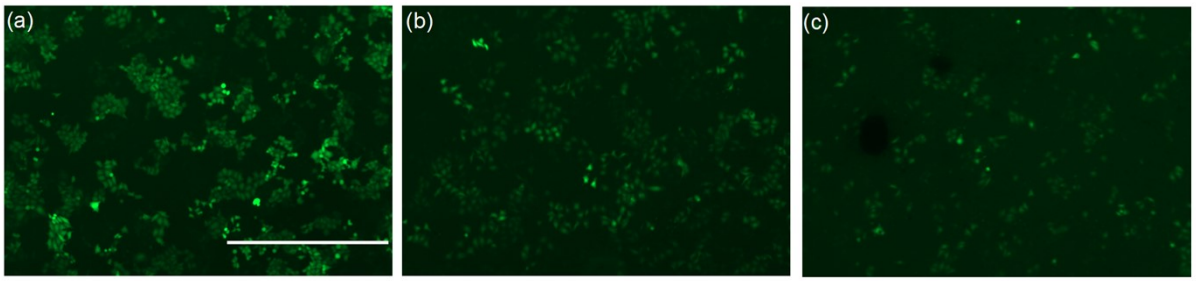
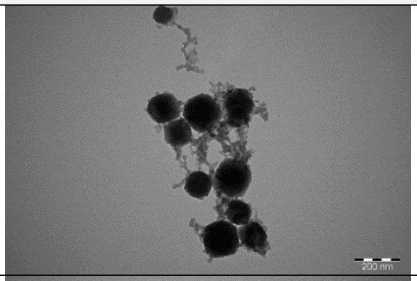
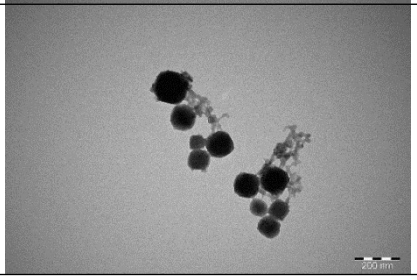


Figure S14. Fluorescence microscopy images of Hela/GFP cells treated with PB@RNP-EuMOFs without (a) and with three times (b) and four times (c) laser irradiation. The scale bar represents 1000 μm.

Table S1 Comparation of bulk-prepared PB@RNP-EuMOFs under different reaction conditions.

Mass ratio of RNP and PB (mg/mg)	Encapsulation Efficiency	PDI	TEM
1:20	25%	0.179	
1:30	36%	0.172	

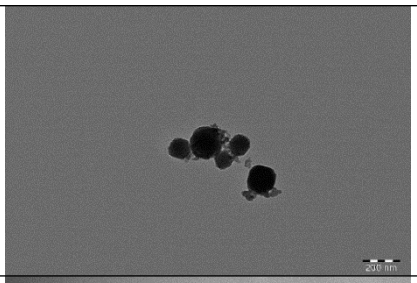
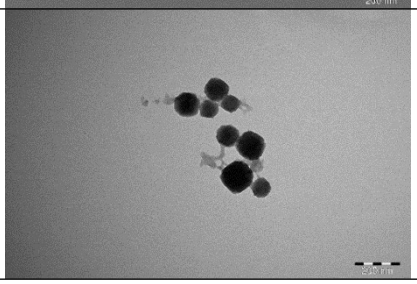
1:60	35%	0.212		
1:80	38%	0.189		

Figure S15. Sanger sequencing results of PCR amplicon by ICE after treatment with PB@RNP-EuMOFs with laser irridation four times. The N indicates a random insertion of a base.