Supporting information

Intracellular activation of bioorthogonal nanozymes through endosomal proteolysis of the protein corona

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1) Synthesis of ruthenium catalyst:

1.1 Synthesis of ligand for Ru catalyst

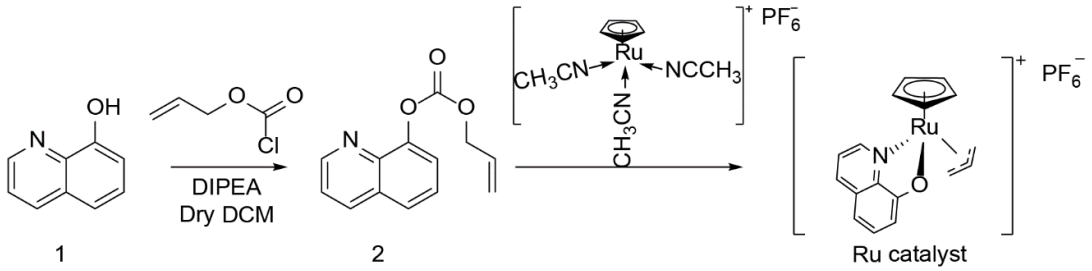

Figure S1. Synthetic scheme of ruthenium catalyst.

8-Hydroxyquinoline (**1**, 435 mg, 3.0 mmol, 1 eq) and N, N-diisopropylethylamine (525 µL, 6.0 mmol, 2 eq) were dissolved in 5 mL dry dichloromethane (DCM) under nitrogen and cooled to 0℃. Allyl chloroformate (385 µL, 3.6 mmol, 1.2 eq) was dissolved in 1 mL of DCM and added dropwise to the stirring solution. The resulting solution was stirred at 0 °C for further 30 minutes and was allowed to stir at room temperature overnight. Once the reaction was completed (checked by TLC), the reaction mixture was washed with water and brine. The organic layer was dried by Na_2SO_4 and concentrated by rotavapor. The crude product was purified by a column using hexane/ethyl acetate 1:1 (Rf: 0.55) as eluent. The solvent was removed by vacuum to obtain **2** as a brownish solid (yield 605 mg, 83%). ^{1}H NMR (400MHz, $CDCl_3$, TMS): δ(ppm): 8.96-8.94 (q, 1H) 8.20-8.18 (q, 1H), 7.78-7.72 (m, 1H), 7.56-7.51 (m, 2H), 7.47-7.43 (q, 1H), 6.10-6.00 (m, 1H), 5.50-5.44 (m, 1H), 5.35-5.32 (m, 1H), 4.82-4.80 (m, 2H). 13C NMR (400MHz, CDCl3): δ(ppm): 153.67, 150.59, 147.27, 141.09, 135.94, 131.28, 129.50, 126.15, 126.13, 121.87, 120.86, 119.18, 69.40.

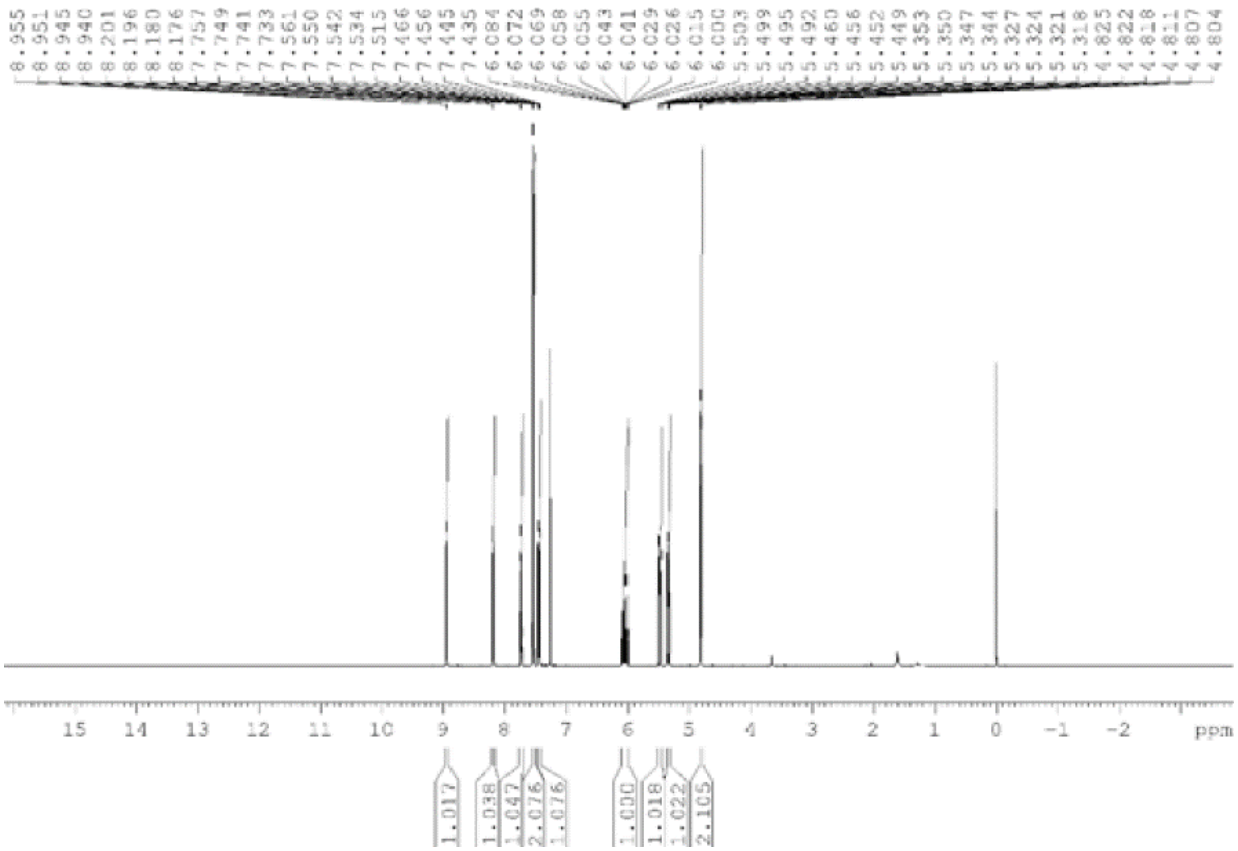

Figure S2. ^{1}H-NMR of the ligand for Ru catalyst.

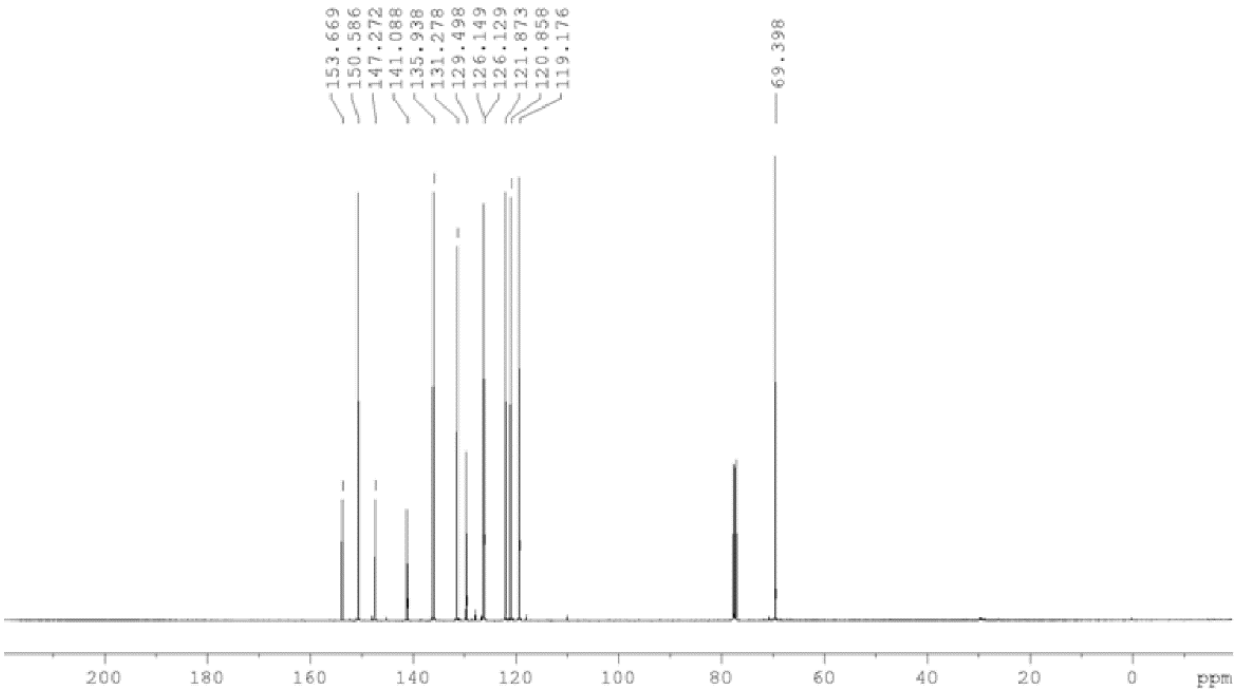

Figure S3. ^{13}C-NMR of the ligand for Ru catalyst.

1.2 Preparation of Ru catalyst solution

8 mg of tris(acetonitrile)cyclopentadienylruthenium(II) hexafluorophosphate complex (1 eq) was dissolved in 1 mL of dry acetonitrile under nitrogen, and 4.4 mg of **2** (1 eq) in acetonitrile was added dropwise. After 1 hour of stirring, acetonitrile was totally removed under reduced pressure and washed three times with ethyl acetate.[1] This Ru catalyst was dried by high vacuum overnight and re-dispersed in 2 mL of acetone as stock solution.

2) 2 nm gold nanoparticles (AuNPs) synthesis:

2.1 Gold core synthesis

Gold nanoparticles (AuNPs) were synthesized by Brust-Schiffrin two-phase method.[2, 3] In brief, 1 g of $HAuCl_4$ was dissolved in 150 mL of water and 150 mL toluene. 2.1 g of tetraoctylammonium bromide (TOAB) was added at maximum stirring speed. Pantanethiol was added dropwise until it turned into white (~ 0.7 ml). Then 2.0 g of sodium boronhydrite was dissolved in 8 mL of water and added into the white solution immediately. After 5 h of stirring, organic layer was collected and dried under reduced pressure at room temperature. The AuNPs core was dispersed in hexane and washed with acetonitrile until all TOAB were fully removed.

2.2 Synthesis of ligand for gold nanoparticles (AuNPs)

All AuNPs are synthesized from same batch of pentanethiol coated AuNPs followed by ligand exchange reactions. Ligands for NP1-3 were synthesized by following previous researches.[4-6]

2.3 Synthesis of functionalization of AuNPs

40 mg of AuNPs core was dissolved in 4 mL of DCM under Argon. 120 mg of ligand in 4 mL DCM/MeOH mixture (v / v = 1:1) was dropwise added to AuNPs core solution and stirred at room temperature for 72 hours. Then, organic solvents were removed under reduced pressure and functionalized AuNPs were washed three times with hexanes and hexanes/DCM mixture (1:1 v/v) separately. These AuNPs was dispersed in MilQ water and dialyzed with skin membrane (10,000 MWCO) for 3 days. Finally, AuNPs was filtered with 0.25μM PES membrane and concentrated with molecular cut off filtration (10,000 MWCO for five times).

3) Characterization of AuNPs and nanozymes

The hydrodynamic diameter and zeta potential of AuNPs and nanozymes were measured by dynamic light scattering (DLS) in DI water using a Malvern Zetasizer Nano ZS instrument by using the measurement angle of 173° (backscatter). Transmission Electron Microscopy (TEM) images of samples were prepared by placing 10 μl of the desired AuNPs and nanozymes solution (1 μM) onto a 300-mesh Cu grid-coated with carbon film. Then the samples were analyzed and photographed using JEOL CX-100 electron microscopy. DLS and TEM images showed that there is no aggregation or change of morphology before and after catalyst encapsulation.

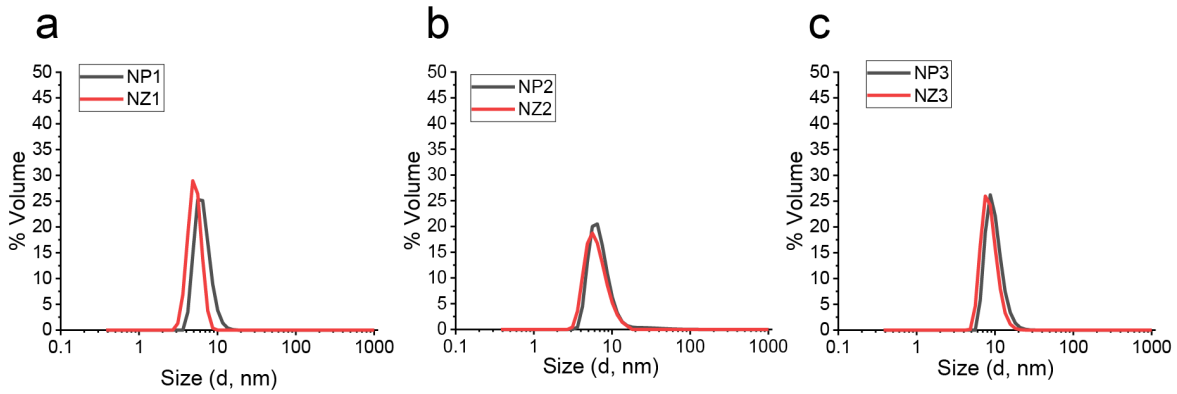

Figure S4. DLS measurements of AuNPs before (NP1, NP2 and NP3) and after (NZ1, NZ2, and NZ3) encapsulation of Ru catalysts.

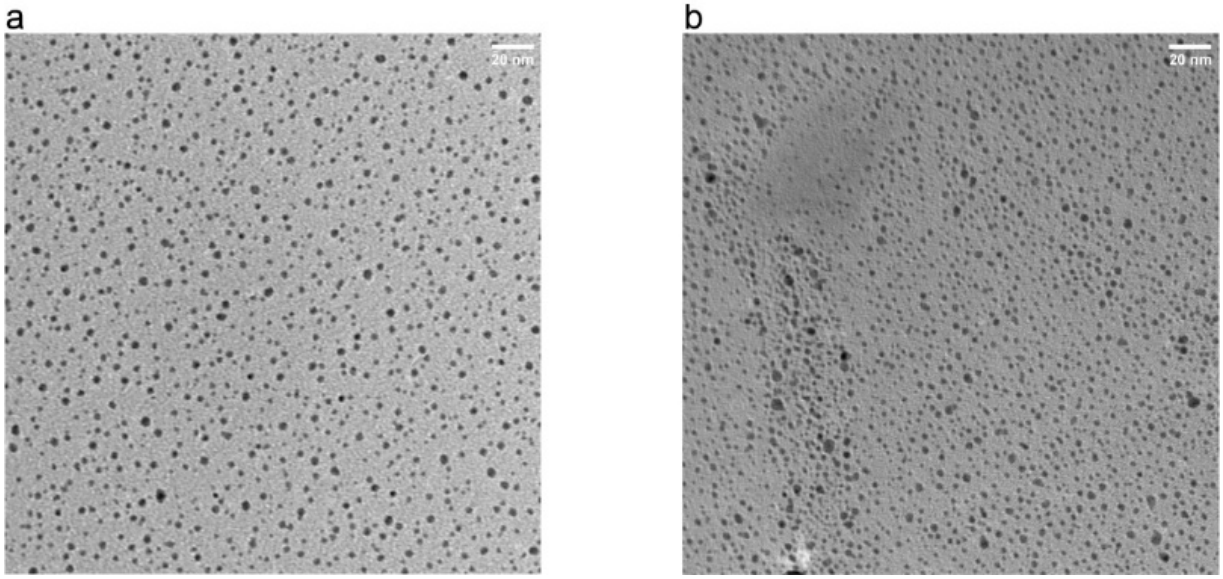

Figure S5. TEM images of **a.** NP1 and **b.** NZ1 with the encapsulation of Ru catalysts.

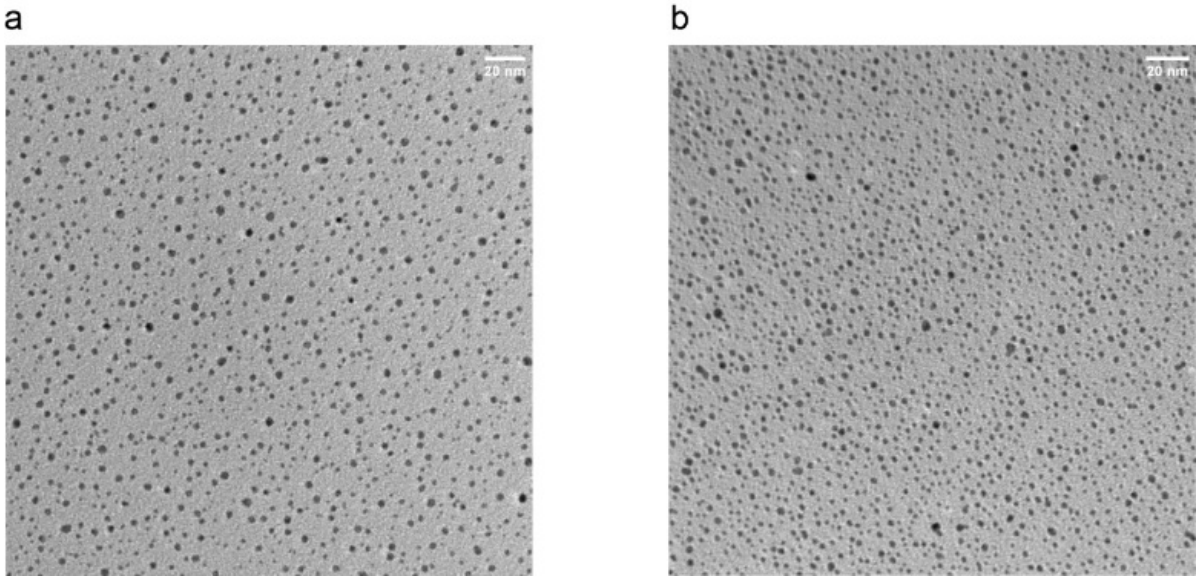

Figure S6. TEM images of **a.** NP2 and **b.** NZ2 with the encapsulation of Ru catalysts.

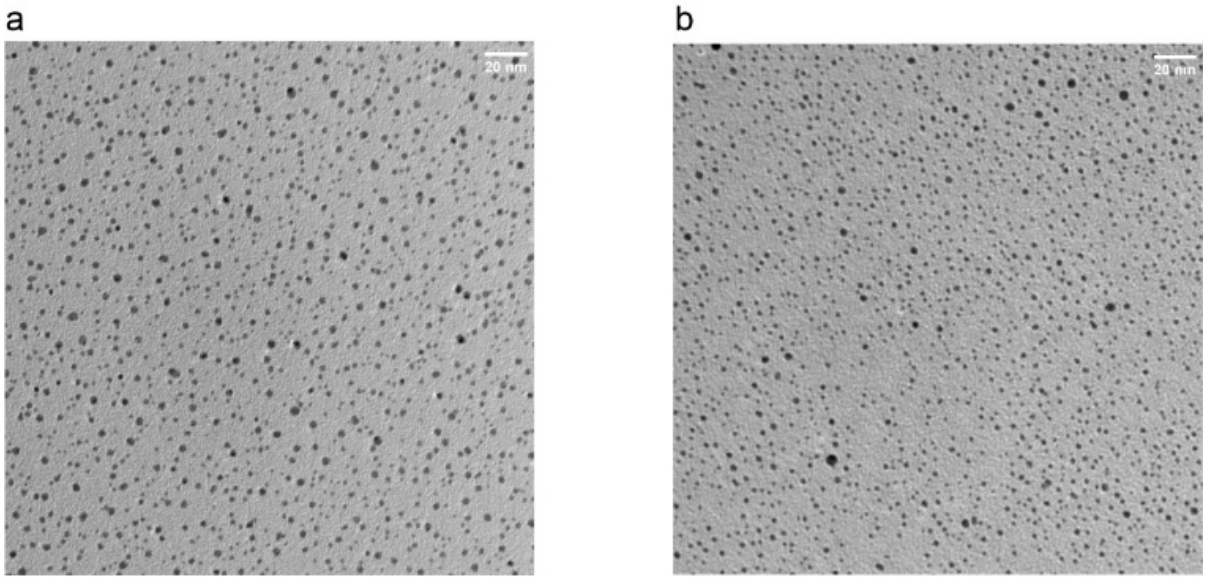

Figure S7. TEM images of **a.** NP3 and **b.** NZ3 with the encapsulation of Ru catalysts.

4) Synthesis of pro-Rhodamine 110

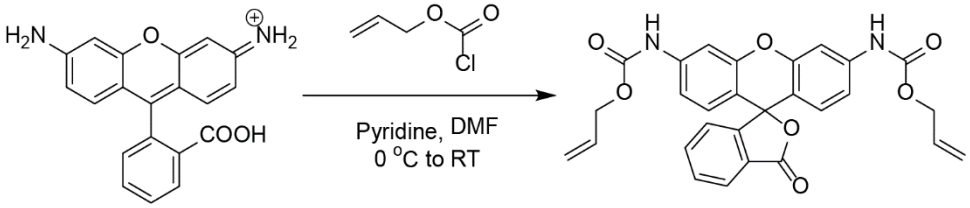

Figure S8. Synthetic scheme of pro-Rhodamine 110.

As been reported,[7] Rhodamine 110 (100 mg, 0.273 mmol, 1 eq) and pyridine (70 µL, 0.867 mmol, 3.2 eq) was dissolved in dry DMF and cooled to 0 °C. Then, allyl chloroformate was dropwise to the solution and the resulting solution was stirred at 0 °C for further 1h followed by warming up to room temperature while stirring overnight. Thereafter, the solution was diluted with water and extracted by ethyl acetate. The organic layer was washed with brine for three times and dried by sodium sulfate. Organic solvent was removed by rotavapor and applied on a short silica column by hexanes/ethyl acetate 2:1 as eluent. The solvent was removed by vacuum to obtain pro-Rhodamine 110 as slightly pinkish white solid (34 mg, yield = 25%).^{1}H-NMR (400 MHz, DMSO-d6) 10.05 (s, 2H), 8.0 (d, 1H), 7.77 (t, 1H) 7.7 (t, 1H), 7.55 (s, 2H), 7.24 (d, 1H), 7.14 (d, 2H), 6.69 (d, 2H), 5.8 (m, 2H), 5.35 (d, 2H), 5.22 (d, 2H), 4.61 (d, 4H).

5) Quantification of Ru catalyst per AuNPs in the nanozymes

ICP-MS analyses were performed on a PerkinElmer nexion 300X ICP mass spectrometer to quantify ^{197}Au and ^{101}Ru. Operating conditions were as follows: nebulizer flow rate: 0.95 L/min; rf power: 1600 W; plasma Ar flow rate: 18 L/min; dwell time: 50 ms. A series of solutions with gold and ruthenium (concentration: 0, 0.2, 0.5, 1, 2, 5, 10, and 20 ppb) were prepared for drawing the calibration curve. Nanozyme solutions were diluted in water to 200 nM. 10 µL sample solution

was transferred to 15 mL centrifuge tubes. 0.5 mL of fresh *aqua regia* was added to each sample including the standard samples and was diluted to 10 mL with DI water.

Table S1: Au and Ru amount in the nanozymes using ICP-MS measurements.

	Au [ppb]	Ru [ppb]	Au [nM]	Ru [nM]	NP [nM]	Ru per NP	Average Ru per NP	SD
NZ1-1	657027	11388	3335727	111757	18532	6.03	6.05	0.03
NZ1-2	660871	11539	3355246	113234	18640	6.07		
NZ2-1	595243	11951	3022049	117275	16789	6.99	6.76	0.32
NZ2-2	596526	11202	3028564	109928	16825	6.53		
NZ3-1	720994	12474	3660488	122413	20336	6.02	5.98	0.05
NZ3-2	699889	11964	3553337	117408	19741	5.95		

6) Kinetic study of nanozymes in PBS solution

Allyl carbamate protected Rhodamine 110 (pro-Rho) was used as a substrate to test the catalytic activity of NZs in PBS solutions. The pro-Rho is stable and non-fluorescence at 37 °C (Figure S8). The catalytic reaction was happened in PBS solution with 5 μM of substrate (pro-Rho) and 200 nM of NZs in 96 well black plate. 5 μM of substrate only were used as negative controls. The kinetic result was measured at 37 °C by fluorescence generation (λ_{ex} = 488 nm, λ_{em} = 521 nm, cut off = 515 nm) with a Molecular Devices SpectraMax M2 microplate reader for 30 mins.

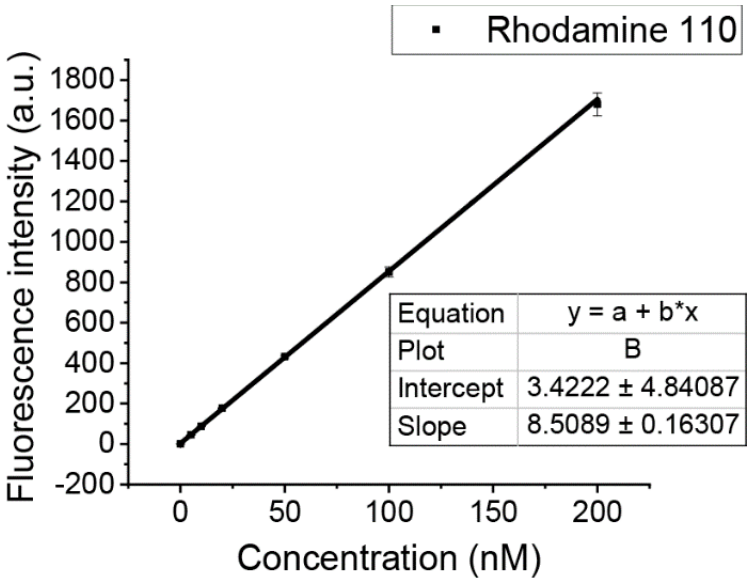

Figure S9. Calibration curve of fluorescence dye (Rhodamine 110) in PBS.

7) Characterization of protein corona formation and its removal on nanozymes by DLS and TEM

NZs were incubated with 1% serum in PBS at 37 °C for 0 h, 0.5 h and 2 h for the formation of protein corona. For the removal of corona, 2 h pre-incubated NZs were incubated with 25 μM for another 30 mins at 37 ℃. The size was measured by DLS.

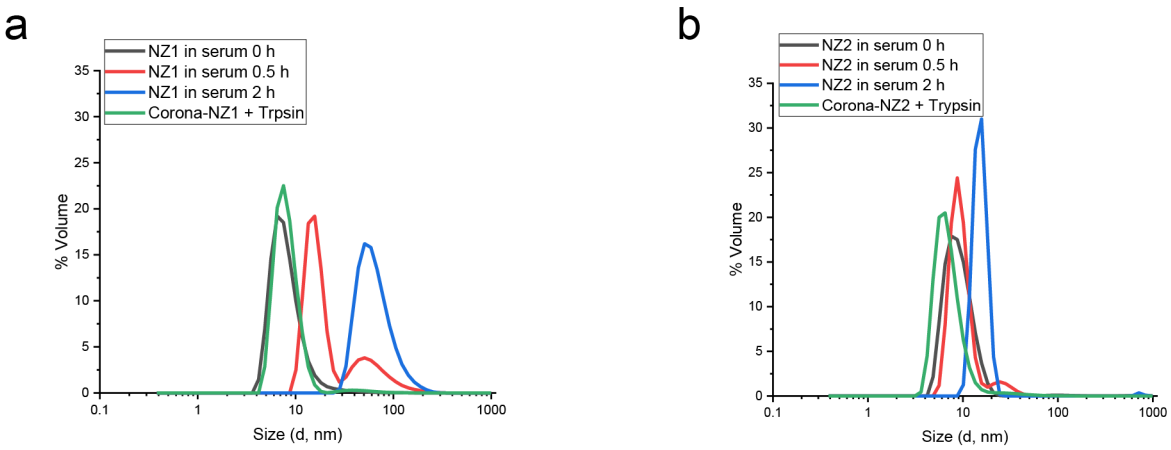

Figure S10. The formation and removal of protein corona on **a.** NZ1 and **b.** NZ2 by DLS.

NZs were pre-incubated with 1% serum in PBS at 37 °C for 2 hours, and protein corona were formed on the surface of nanozymes. For the trypsin study, pre-incubated NZs were further incubated with trypsin (25 μM) for another 30 mins at 37 ℃, and protein corona were removed under proteases. Transmission Electron Microscopy (TEM) samples were prepared by placing 10 μl of each solution onto a 300-mesh Cu grid-coated with carbon film and imaged using JEOL CX-100 electron microscopy.

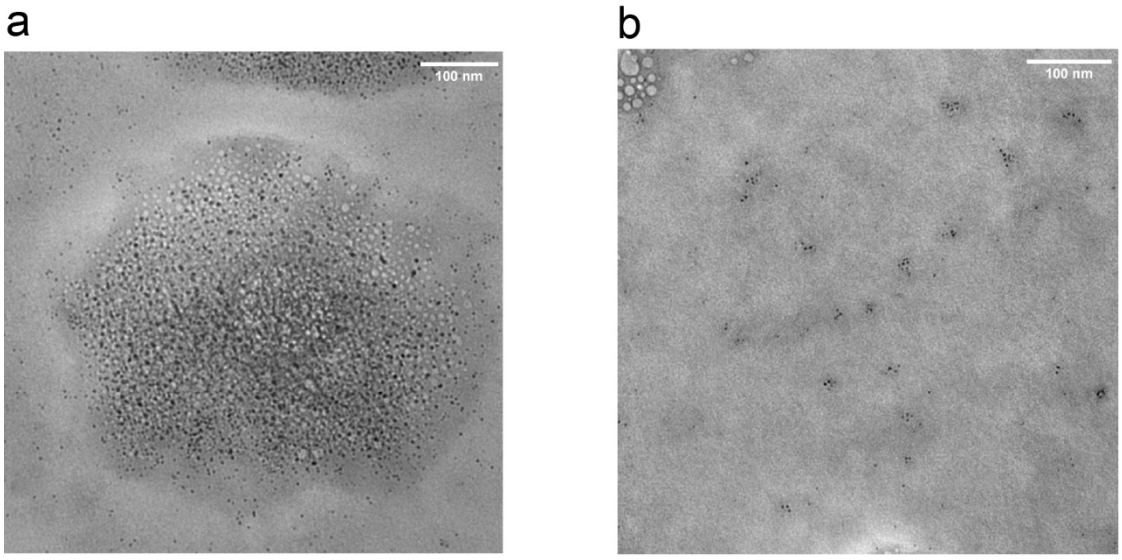

Figure S11. TEM images of corona formation on **a.** NZ1 and **b.** NZ2. NZ1 formed big nanozyme-protein assemblies while NZ2 formed small clusters of nanozymes with proteins. The

size of corona-NZ1 which was bigger than the measurement on DLS was due to the spherical structure collapse during drying process.

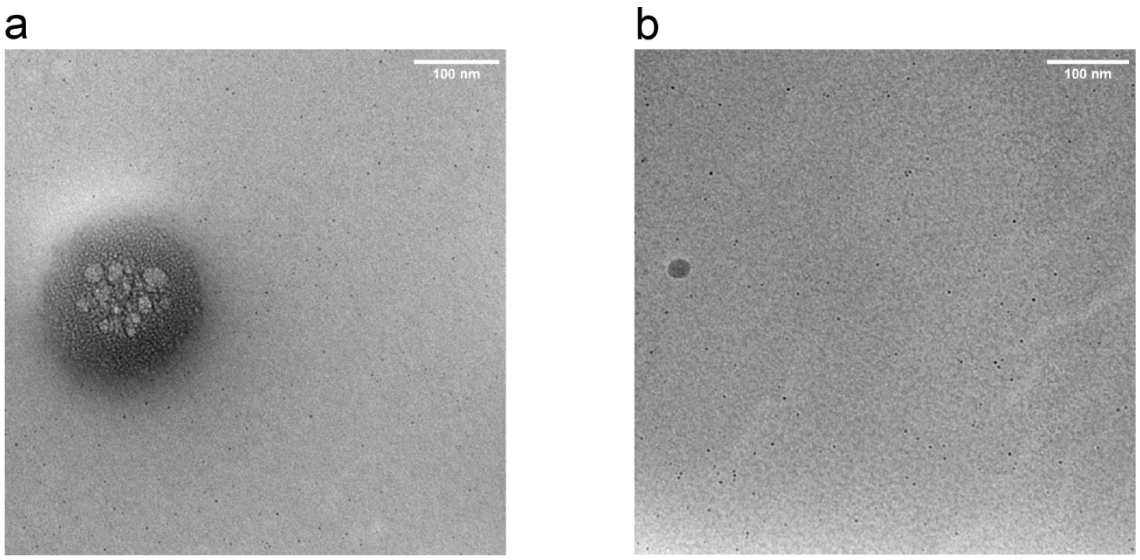

Figure S12. TEM images of corona removal by trypsin on **a.** NZ1 and **b.** NZ2. A significant increase of free nanozymes was observed in both cases.

8) Cellular uptaken and cell viability of nanozymes

HeLa cells were cultured in cell culture flasks using low glucose Dulbecco's modified Eagle medium (DMEM) containing 10% of FBS and 1% of penicillin/streptomycin at 37 °C under 5% CO_2 condition.

For the uptaken study, 20K/well HeLa cells were seeded in 24-well plate prior to the experiment. After 24h, cells were washed three times with PBS and incubated with NZs (200 nM) for 8h at 37 °C. Subsequently, cells were washed with PBS to remove excess NZs, and then subjected to lysis buffer. The lysed cells were then further processed for ICP-MS analysis.

For cell viability study, 20K/well HeLa cells were seeded in 24-well plate prior to the experiment. After 24h, cells were washed three times with PBS and incubated with NZs (200 nM) for 24 h at 37 °C. The cells were then washed three times with PBS buffer, and 10% Alamar Blue in serum-containing media was added to each well and further incubated at 37 °C for 2.5 h. Cell viability was determined by measuring the fluorescence intensity at 570 nm using a SpectraMax M2 microplate spectrophotometer. Each experiment was comprised of 3 replicates.

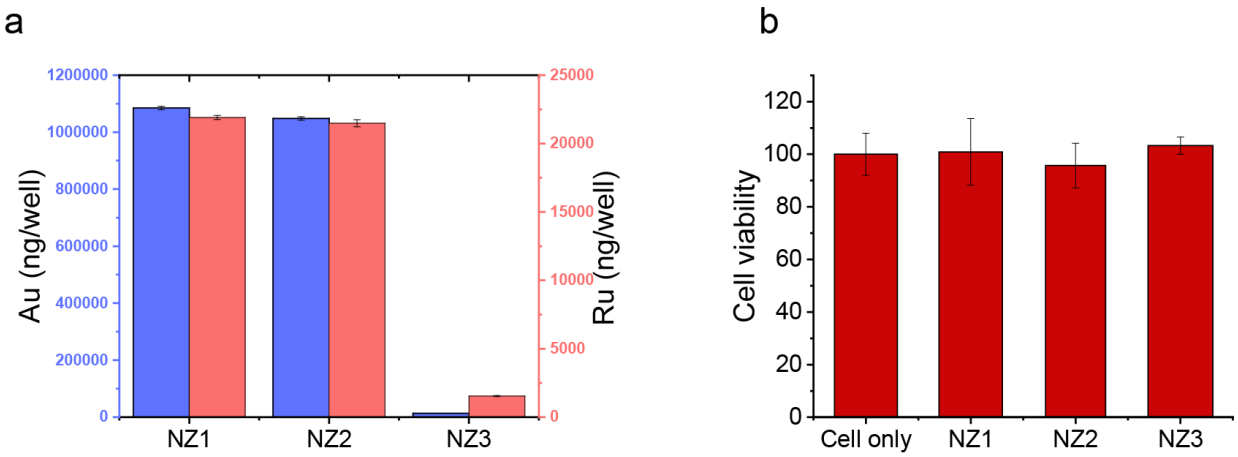

Figure S13. Cellular uptake of Au(ng/well) and Ru (ng/well) in HeLa cells **a.** after 8 h incubation with nanozymes (200 nM) and **b.** cell viability.

References:

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