

Electronic Supplementary Information

Boron ketoiminate-based conjugated polymers with tunable AIE behaviours and their applications for cell imaging

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ESI 1. Measurements and materials

All solvents and reagents were commercially available and analytical-reagent-grade. THF and Et₃N were purified by distillation from sodium in the presence of benzophenone. NMR spectra were obtained using a 400-Bruker for ¹H NMR and reported as parts per million (ppm) from the internal standard TMS. Fluorescence spectra were obtained from a Shimadzu RF-5301PC Spectrofluorometer. Ultraviolet-visible (UV-vis) spectra were obtained using a Perkin-Elmer Lambda 35 spectrophotometer. Time-resolved fluorescence decays were recorded with an Edinburgh Instrument FLS 920 fluorospectrophotometer. Cyclic voltammetry (CV) measurements were performed on a BAS100W. Molecular weight of polymers was determined by GPC with Waters-244 HPLC pump and THF was used as solvent and relative to polystyrene standards. The particle size distributions of CPNs were measured by dynamic light scattering (DLS) using a particle size analyser (BI-200SM, Brookhaven instruments Corp., Holtsville, NY). The morphology of the CPNs were characterized on transmission electron microscope (TEM) (JEOL JEM-200CX, Japan) operated at 200 kV. Confocal laser scanning microscope images of CPNs were taken on a Olympus Fluo-view 1000. Milli-Q water was supplied by Milli-Q Plus System (Millipore Corporation, Bedford, USA). Boron trifluoride etherate was purchased from energy-chemical Co. Ltd and used without further purification.

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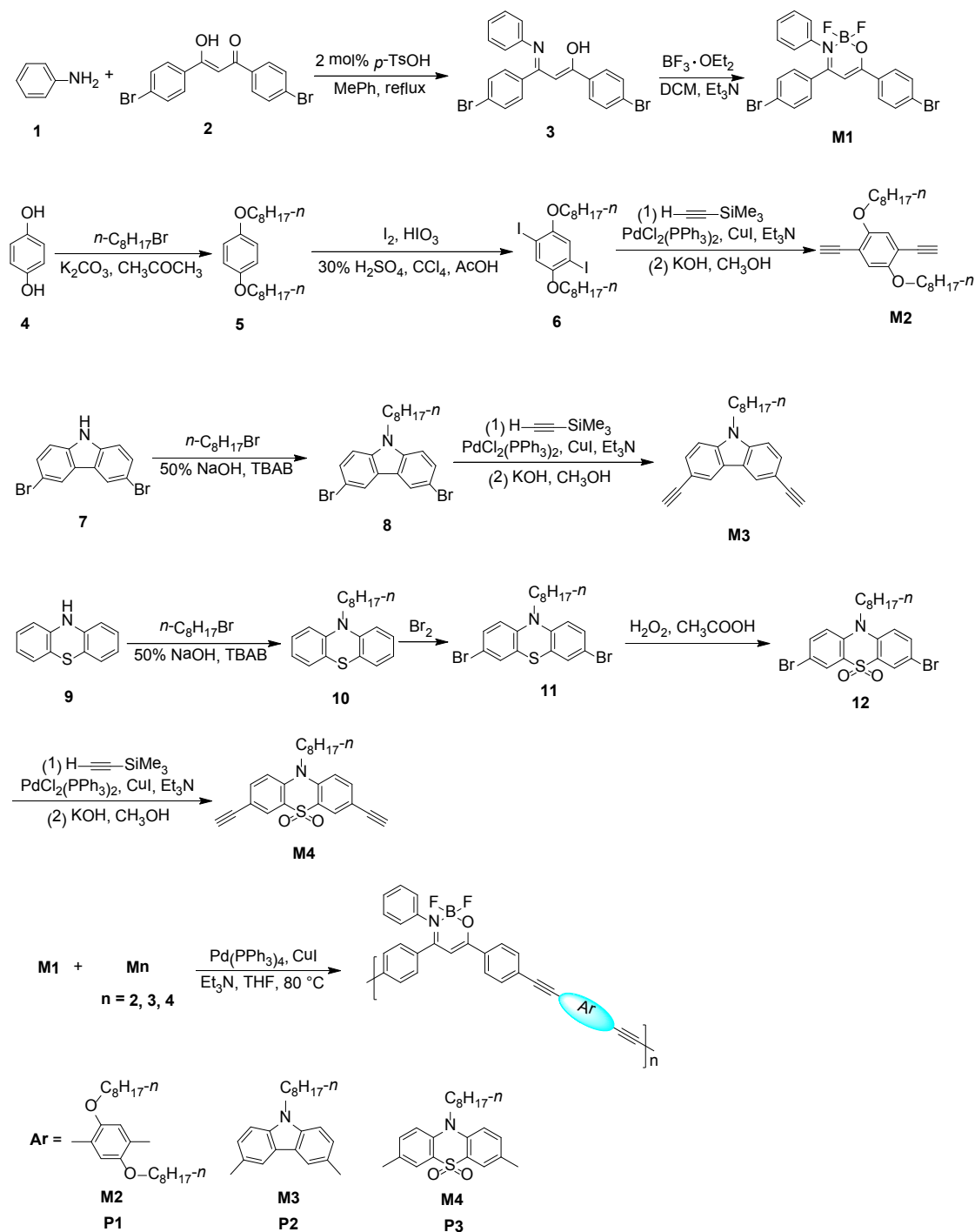
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ESI 2. Synthesis of the monomers and conjugated polymers

The procedures for the monomers **M1-M4** and the boron ketoiminate-based conjugated polymers **P1-P3** are illustrated in Scheme S1. **M1**, 4,6-bis(4-bromophenyl)-2,2-difluoro-3-phenyl-2*H*-1,3,2-oxazaborinin-3-ium-2-uide,¹ **M2**, 1,4-diethynyl-2,5-bis(octyloxy)benzene,² **M3**, 3,6-diethynyl-9-octyl-9*H*-carbazole,³ and **M4**, 5,3,7-diethynyl-10-octyl-10*H*-phenothiazine-*S,S*-dioxide,⁴ were prepared according to the procedure described in the reported literature. The conjugated polymers **P1-P3** were prepared from Pd-catalyzed Sonogashina coupling reaction.



Scheme S1 Synthetic procedures for the monomers **M1-M4** and the conjugated polymers **P1-P3**

Synthesis of P1

M1 (191.3 mg, 0.5 mmol), **M2** (252.5 mg, 0.5 mmol), [Pd(PPh₃)₄] (28.9 mg, 0.025 mmol) and CuI (4.7 mg, 0.025 mmol) were placed in a 50 mL Schlenk tube. The tube was evacuated and refilled with N₂ three times and then 10 mL of THF and 20 mL of Et₃N were added via a syringe. The mixture was stirred at 80 °C under N₂. After 24 h, the mixture was cooled to room temperature and was then filtered off. The filtrate was concentrated under vacuum, and the residue was dissolved in a small quantity of THF. The resulting solution was added dropwise into methanol (50 mL). The polymer was collected by filtration and dried in vacuum to give 251.3 mg as a yellow solid in 69% yield. GPC results: $M_w = 20464$, $M_n = 8210$, PDI = 2.49. ¹H NMR (400 MHz, CDCl₃): δ 8.07–8.03 (m, 2H), 7.67–7.63 (m, 2H), 7.44–7.41 (m, 2H), 7.24–7.13 (m, 5H), 7.05–6.95 (m, 2H), 6.40 (d, 1H), 5.75–5.59 (m, 1H), 4.08–3.93 (m, 6H), 2.08–1.99 (m, 3H), 1.90–1.80 (m, 7H), 1.61–1.47 (m, 9H), 1.38–1.29 (m, 19H), 0.88–0.86 (m, 6H).

Synthesis of P2

P2 was prepared from monomers **M1** and **M3** in a yield of 74% according to the same method with **P1**: yellow solid. GPC results: $M_w = 11061$, $M_n = 4220$, PDI = 2.62. ¹H NMR (400 MHz, CDCl₃): δ 8.32–8.24 (m, 1H), 8.09–8.04 (m, 2H), 7.68–7.64 (m, 4H), 7.48–7.40 (m, 4H), 7.25–7.13 (m, 6H), 6.40 (d, 1H), 4.29 (s, 2H), 1.86 (d, 2H), 1.34–1.25 (m, 12H), 0.88–0.85 (t, 3H).

Synthesis of P3

P3 was prepared from monomers **M1** and **M4** with a yield of 79% according to the same method with **P1**: yellow solid. GPC results: $M_w = 10352$, $M_n = 4750$, PDI = 2.18. ¹H NMR (400 MHz, CDCl₃): δ 8.33–8.28 (m, 1H), 8.05 (d, 2H), 7.78–7.76 (m, 1H), 7.65 (d, 2H), 7.43 (d, 2H), 7.35 (d, 1H), 7.25–7.21 (m, 2H), 7.16–7.13 (m, 3H), 6.42 (d, 1H), 4.20–4.12 (m, 1H), 4.00–3.91 (m, 1H), 2.08–1.81 (m, 4H), 1.60–1.50 (m, 6H), 1.41–1.25 (m, 9H), 0.90–0.86 (m, 3H).

ESI 3. Preparation and characterization of CPNs

The CPNs were prepared *via* reprecipitation method.⁴ In brief, 2 mL of conjugated polymer **P1** in THF solution (0.01 mg/mL) was injected rapidly into 8 mL MilliQ water under sonication. The mixture was further concentrated by evaporation under reduced pressure, followed by an additional filtration step with a 0.22 μ m syringe filter to give the CPNs. **P2** and **P3** NPs were prepared following the same procedure. The particle size and size distribution of CPNs were determined by laser light scattering with a particle size analyzer (90 Plus, Brookhaven Instruments Co. USA) at a fixed angle of 90° at room temperature. The morphologies of **P1–P3** NPs was characterized at a JEOL JEM-200CX transmission electron microscope (TEM) operated at 200 kV. The samples were prepared by dropping the solution onto copper mesh carbon-coated copper grid following negative staining with 2.0% (w/v) phosphotungstic acid and dried in a vacuum. All experiments were performed under ambient conditions in air.

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2. Ma X.; Jiang X.; Zhang S.; Huang X.; Cheng Y.; Zhu C. *Polym. Chem.* **2013**, 4, 4396–4404.

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ESI 4. Cell culture

HeLa cells were cultured in Dulbecco's modied Eagle's medium (DMEM) containing 10% fetal bovine serum and antibiotics (100 units per mL penicillin and 100 $\mu\text{g mL}^{-1}$ streptomycin), maintained at 37 °C in a humidied atmosphere of 95% air and 5% CO₂. Before the experiment, the cells were pre-cultured until confluency was reached.

ESI 5. Photostability of CPNs

The photostability of **P1-P3** NPs was investigated by monitoring their respective fluorescence intensity changes in a phosphate buffer solution (PBS) at 37 °C by using confocal microscopy. The CLSM images of each sample were recorded at 2 min interval under continuous laser scanning at an excitation wavelength of 405 nm with 5 mW laser power. The fluorescence intensity of each image was analyzed by Image Pro Plus software, and was further expressed by I/I_0 , where I_0 is the initial fluorescent intensity of fresh CPNs suspension and I is that of CPNs suspension after continuous laser scanning.

ESI 6. Cytotoxicity assay

The cytotoxicity of CPNs against HeLa cells was evaluated by MTT assay. Briefly, HeLa cells were seeded in 96-well plates (Costar, IL, USA) at an intensity of 4×10^4 cells mL^{-1} . After 24 h incubation, the cells were exposed to a series of doses of CPNs with the concentration of 0, 5, 10, 20, and 40 $\mu\text{M/L}$ at 37 °C. After 48 h, the sample wells were washed twice with 1×PBS buffer and 100 mL of freshly prepared MTT (0.5 mg mL^{-1}) solution in culture medium was added into each sample well. The MTT medium solution was carefully removed after 3 h incubation in the incubator for the sample wells, whereas the control wells without addition of MTT solution were washed twice with 1×PBS buffer. DMSO (150 μL) was then added into each well and the plate was gently shaken for 10 min at room temperature to dissolve all the precipitates formed. The absorbance of individual wells at 570 nm was then monitored by the microplate Reader (BioTek, PowerWave XS2, Vermont, USA). The absorbance of MTT in the sample well was determined by the differentiation between the absorbance of the sample well and that of the corresponding control well. Cell viability was expressed by the ratio of the absorbance of MTT in the sample wells to that of the cells incubated with culture medium only.

ESI 7. Cell staining and imaging

A fresh stock of HeLa cells was seeded into a glass bottom dish with a density of 1×10^5 cells per dish, and incubated for 24 h. Subsequently, the solution was then removed, and the adherent cells were washed with PBS buffer (2 mL×3). Afterwards, the cells were coincubated with 4 μM CPNs

suspension for 2 h at room temperature. The solution was then removed, and the cells were washed with PBS (2 mL×3) before observation. For confocal imaging, the cell suspension was dropped into the chambers (LAB-TEK, Chambered Coverglass System) and imaged immediately with a confocal laser-scanning microscope (Olympus, FV-1000; λ_{ex} = 405 nm; Fluorescent signals were collected at 500–600 nm).

ESI 8. ^1H NMR spectra of P1-P3 in CDCl_3

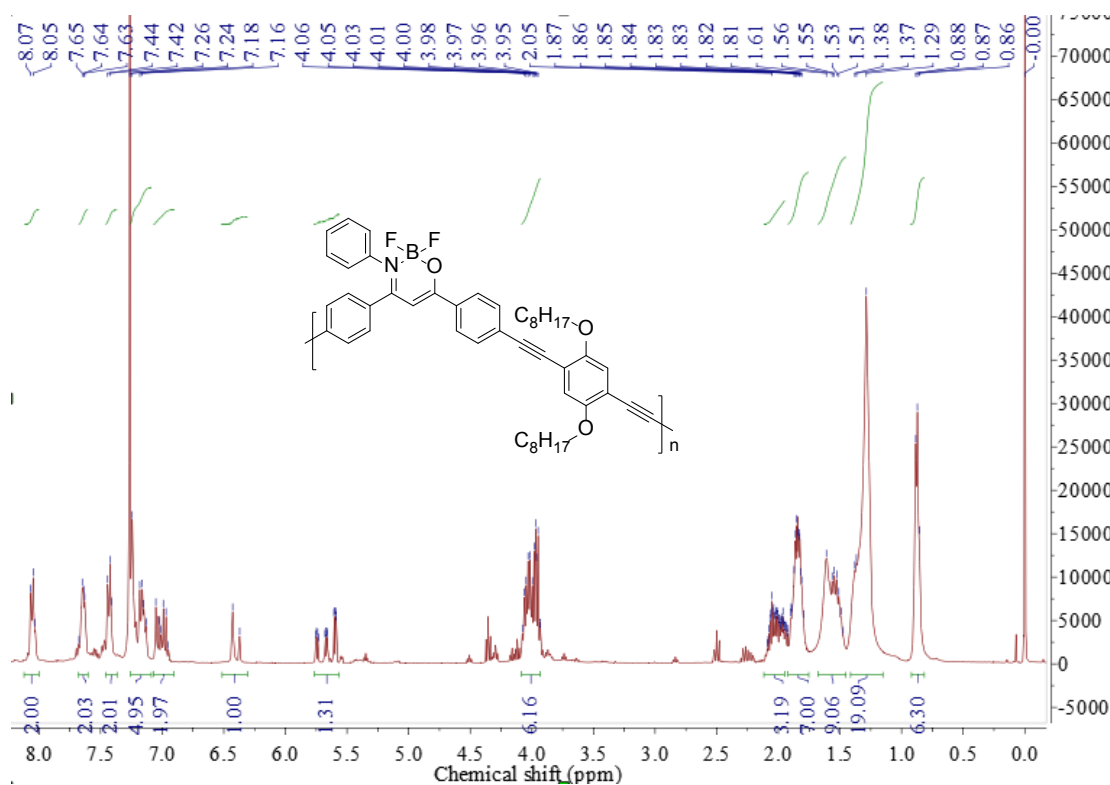
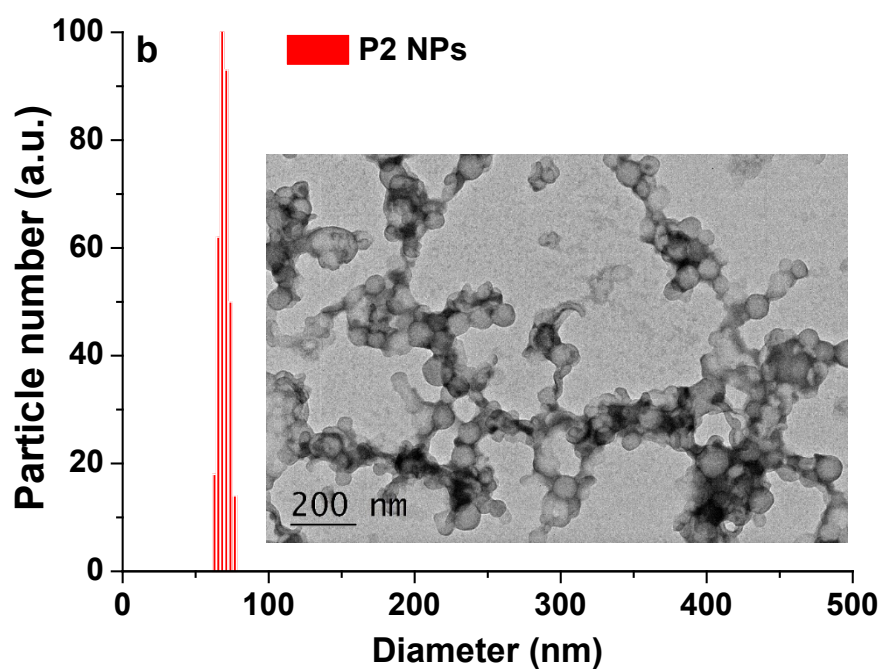
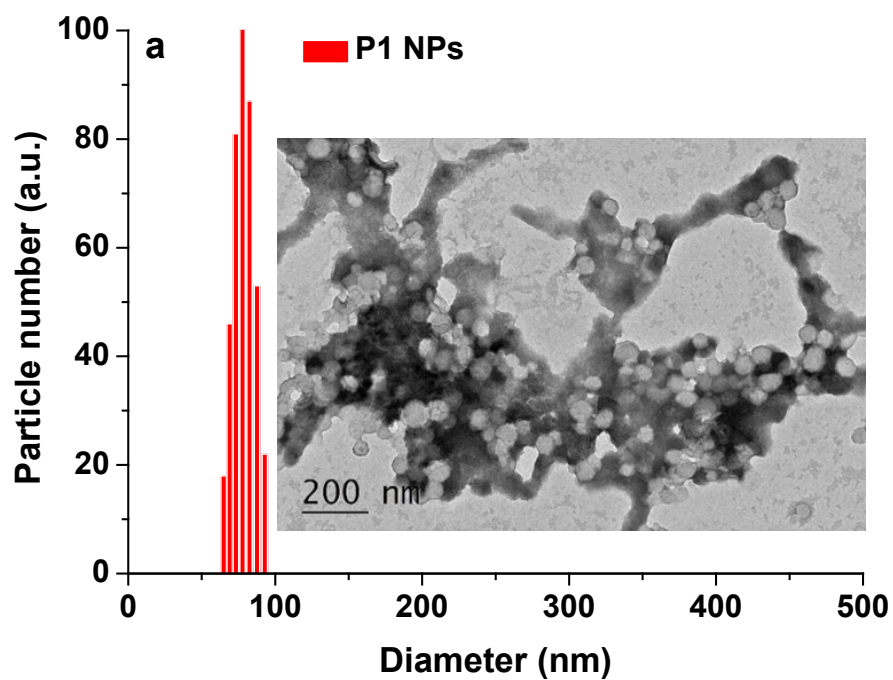


Figure S1. ^1H NMR of P1 in CDCl_3



ESI 9. Particle size and morphology characterization of P1-P3 NPs



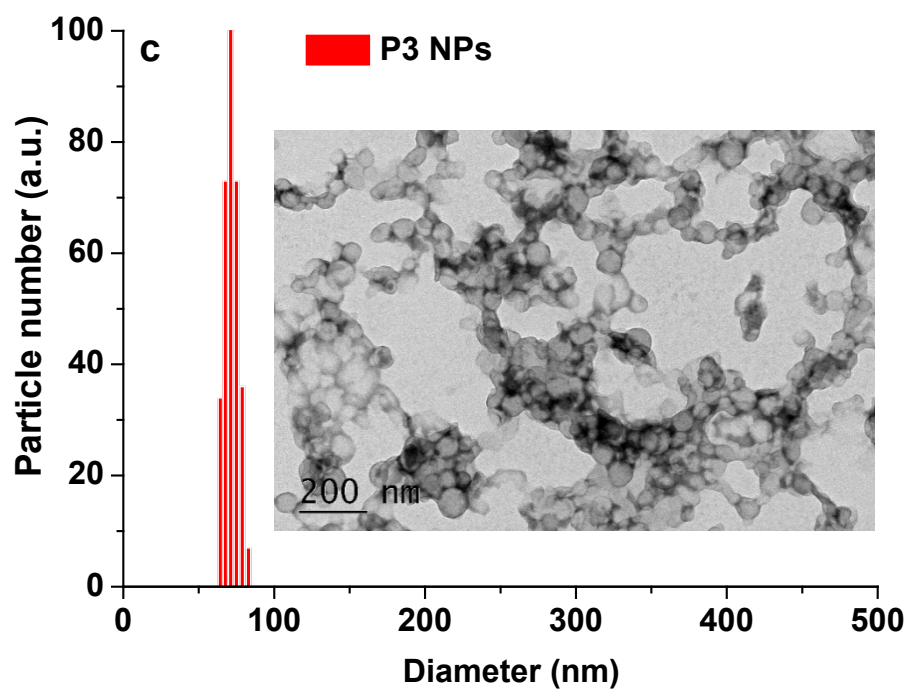


Figure S4. Histograms of the size distribution (measured by DLS) of **P1-P3** NPs (a-c). Inset shows TEM images of **P1-P3** NPs.

ESI 10. The fluorescence emission lifetime and mean square deviation χ^2 (CHISQ) of CPNs

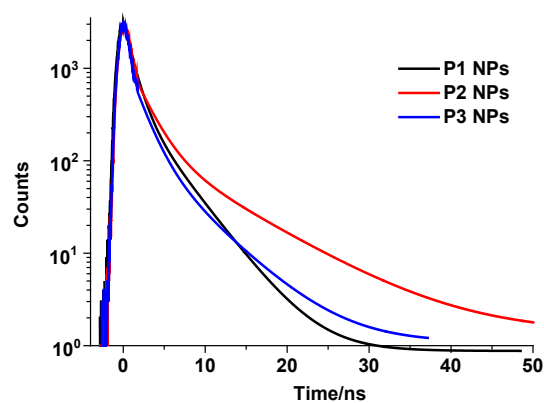


Figure S5. Fluorescence decay curves of **P1-P3** NPs suspension (10 μ M, all fluorescence lifetime measurements were obtained at an excitation from a laser tuned to $\lambda_{\text{ex}} = 405$ nm).

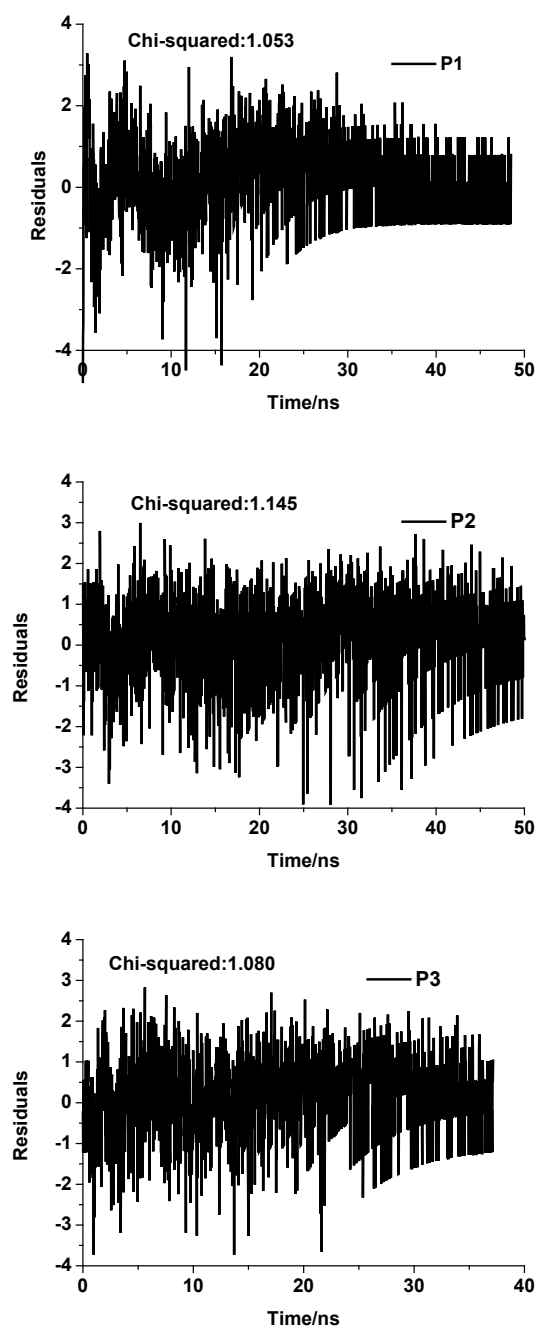


Figure S6. The mean square deviation χ^2 (CHISQ) of **P1-P3** NPs