Supplementary Material (ESI) for Nanoscale

Encapsulated enhanced green fluorescence protein in silica nanoparticle for cellular imaging

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Experimental

1. Chemicals and Materials

Tetraethoxysilan (TEOS, GR), (3-aminopropyl) trimethoxysilane (APTS, GR), Triton X-100 (GR), urea (GR) and proteinase K were purchased from Sigma. Cyclohexane (AR), n-hexanol (AR), acetone (AR), guanidine hydrochloride (GdHCl) (AR) and ammonia solution (GR) were obtained from Sinopharm Chemical Reagent Co., Ltd. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC•HCl) and N-hydroxysuccinimide (NHS) were obtained from GL Biochem (Shanghai) Ltd. RPMI 1640 culture medium was obtained from Genom Biomedical Technology Co.Ltd.(Shanghai), and fetal bovine serum was obtained from Lanzhou National Hyclone Bio-Engineering Co. Ltd (China). EGFP was obtained as reported previously [1].

2. Covalently Encapsulated EGFP in Silica Nanoparticle

Firstly, purified EGFP was functionalized with APTS by the conventional EDC/NHS method. Briefly, 1 mL of 10 mg/mL EGFP was mixed with 2 mL of 50 mM phosphate buffer (pH 7.5). Then 40 mg EDC•HCl and 50 mg NHS were added to the mixture and stirred for 30 min. After that, 500 μL of APTS was added, and the mixture was stirred vigorously at the room temperature for 24 hours to complete the reaction. The APTS-functionalized EGFP (APTS-EGFP) was purified on a G-25 column and then concentrated by ultra-filtration on Millipore Amicon Ultra-50 filters.

The APTS-EGFP was then encapsulated in silica NP following the common reverse micro-emulsion method. Typically, the water-in-oil microemulsion was prepared by mixing cyclohexane (7.50 mL), Triton X-100 (1.77 mL), n-hexanol (1.80 mL), concentrated APTS-EGFP solution (300 μL), and TEOS (100 μL). Then, 25% ammonia was added to the system to initiate the polymerization. After stirring the mixture for 24 hours for the completion of the polymerization, acetone (20 mL) was added to break up the microemulsion and precipitate the EGFP@silica NPs. The NPs were obtained by centrifugation at 12000 rpm and washed several times with ethanol and deionized water. The particle size of EGFP@silica was easily controlled by change the volume of 25% ammonia added, the volumes for the samples shown in Fig. 2 a, b, c. and d are 20, 30, 50 and 100 μL, respectively.

The nitrogen content of the NPs was determined by a Vario EL-III elemental analyzer (Elementar, German) to calculate the protein content in the EGFP@silica NPs.

All fluorescence spectra were recorded on a F7000 spectrophotometer (Hitachi, Japan) at 25°C. For the accurate comparison between free EGFP and encapsulated EGFP, 395 nm was chosen as the excitation wavelength to avoid Rayleigh scattering, and the sample concentration was adjusted so that its absorbance at 395 nm was less than 0.1. The encapsulation efficiency of EGFP was determined according to the remaining EGFP fluorescence in the reaction mixture after removing the formed NPs by centrifugation. For accurate calculation, the fluorescence spectrum of the total EGFP in the same reaction mixture without TEOS was recorded.

The leakage of EGFP from the silica NPs was determined according to the retained fluorescence intensity of the NPs after each washing cycles.

Protease K assays for EGFP were carried out in 6 M urea and 50 mM Tris-HCl buffer (pH 7.5) at 58 °C with the final concentration of protease K of 0.1 $mg \cdot mL^{-1}$. At predetermined time points, aliquots of sample were removed for the fluorescence measurement.

TEM images were obtained on a JEM 200CX microscope (JEOL, Japan). The samples were prepared by drop-coating onto a carbon-coated 300 mesh copper grid and dried under room temperature

3. Cellular imaging by EGFP@silica NPs

The murine macrophage cell line RAW264.7 cells were cultured in RPMI 1640 culture medium with 10% (v/v) fetal bovine serum. Approximately 3×10^5 RAW264.7 cells were seeded in the culture dishes (diameter 35 mm) and cultured using the same culture medium (2 mL per dish) at 37 °C under 5% CO_2/95% air. After cells were incubated for 24 h, EGFP@silica NPs were introduced to the cells with a final concentration of about 0.4 mg/mL in culture medium and cultured for 5 h. Then, the cells were washed three times with 1 mL D-hanks solution and kept in culture medium (1 mL) for the Confocal microscopy (Olympus FM 1000, Japan) analysis. λ_{EX}=488 nm, detection in the 500-600 nm range.

Reference

1. A. Cao, Z. Ye, Z. Cai, E. Dong, X. Yang, G. Liu, X. Deng, Y. Wang, S.-T. Yang, H. Wang, M. Wu and Y. Liu, *Angew. Chem. Int. Ed.*, 2010, **49**, 3022.