Supporting Information

Fluorescent Core-shell Nanoparticle for Specific Cell Nucleus Staining

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Materials
CuBr (Aldrich, 99.999%), 4,4’-di-tert-butyl-2,2’-bipyridine (DTB-bipy) (Aldrich, 98%) and 2-butanone (ARCOS, 99%) were used as obtained. PDI-labeled first-generation dendrimer bearing eight 2-bromo-2-methylpropionic ester groups (1) and PDI-labeled second-generation dendrimer bearing sixteen 2-bromo-2-methylpropionic ester groups (3) were synthesized as reported.[1] 2- tert-Butylacrylate (ARCOS, 99%) was rinsed with 5% NaOH, followed with water and dried over CaCl₂. It was then distilled under reduced pressure from CaCl₂ and stored under nitrogen at -20 °C.

ATRP of tert-butylacrylate (tBA)
All polymerizations were performed in a Schlenk apparatus. The reaction mixtures (tert-butylacrylate: Br in PDI derivative: copper bromide: DTB-bipy = 400:1:1:1, molar ratio) in 2-butanone (1 g tBA/1ml solvent) were degassed by three freeze-pump-thaw cycles and placed in a thermostated oil bath maintained at 80 °C prior to the polymerization. After a specific polymerization time (4 h for 2, 6 h for 4) the reaction mixtures were cooled down to room temperature, and the content was diluted with THF and passed through a column of neutral alumina to remove the copper salts. The polymers were precipitated from an excess of
methanol, filtered, and dried under vacuum to give products 2 (Conversion: 12.5%, $M_n,GPC = 48800$ g/mol, $PD=1.15$; $M_n,NMR=57600$ g/mol, calculated from $^1H$-NMR spectra of product 2 in S-Figure 1) and 4 (Conversion: 20%, $M_n,GPC = 153000$ g/mol, $PD = 1.25$; $M_n,NMR=174000$ g/mol, calculated from $^1H$-NMR spectra of product 4 in S-Figure 1) as red powders.

Hydrolysis of PRBA in core-shell nanoparticles

The PRBA polymer (2 or 4, 100 mg) was placed in a Schlenk flask equipped with a magnetic stirring bar. Then, the flask was evacuated and backfilled with argon three times. Dichloromethane (20 ml) was added to dissolve the polymer. Then, trifluoroacetic acid (10 ml) was added to the solution, and the mixture was stirred at room temperature for 20 h. The solvent was removed from the resulting heterogeneous mixture, and the residual solid was washed with dichloromethane (10 ml, 3 times) followed by drying under vacuum at room temperature for 10 h to give P1 or P2 as red solids.

S-Scheme 1. Synthesis of PDI labeled core-shell nanoparticle P2. i: tert-butyl acrylate, CuBr, DTB-bipy, 2-butanol, 100°C. ii: CF$_3$COOH, CH$_2$Cl$_2$, RT.
S-Figure 1. $^1$H-NMR spectra of products 2 (in CD$_2$Cl$_2$), 4 (in CD$_2$Cl$_2$), P1 (in D$_2$O) and P2 (in D$_2$O); Note: By comparing $^1$H-NMR spectra of P1 (or P2) to that of 2 (or 4), one can clearly observe that the methyl groups from tert-butyl protective groups have been completely removed.

S-Figure 2. FT-IR spectra of final products P1 and P2. Note: One can observe the typical peaks of –COOH groups in the region of 2500-3500 cm$^{-1}$ and 1700 cm$^{-1}$.
Fluorescence quantum yield of the mixture of histones and P1

The relative fluorescence quantum yield of the mixture of histones and P1 was calculated using the following equation:

\[ Q = Q_R \frac{I}{I_R} \frac{OD_R}{OD} \frac{n^2}{n_R^2} \]

where \( Q \) is the quantum yield, \( I \) is the integrated emission intensity, \( n \) is the refractive index, and \( OD \) is the optical density. The subscript \( R \) refers to the reference fluorophore of known quantum yield. Herein, Cresyl Violet in methanol (\( \Phi_f = 0.54 \))\(^{[2]} \) was used as a reference chromophore.

*Drosophila* stocks

All fly stocks are described at [http://flybase.bio.indiana.edu](http://flybase.bio.indiana.edu). The cell membrane marker CD8-GFP was expressed by the Gal4-UAS system\(^{[3]} \) using the Gal4-driver C765-Gal4. Larvae were reared at 25°C.

Histochemical staining

*Drosophila* third instar larvae were dissected and fixed according to standard protocols. Dissected larvae were incubated in 0.01% P1 buffer solution for 1h rotating at room temperature, then washed for 4×15min. Immunohistochemistry was performed as previously described\(^{[4]} \). Primary antibodies used were rabbit anti-GFP (1:2000) (Clontech), rabbit anti-histone H4 (1:100) (Santa Cruz) and mouse anti-\( \alpha \)-tubulin (1:1000) (Sigma). Secondary antibodies used were donkey anti-mouse or anti-rabbit IgG-FITC (1:100 dilution) (Jackson Immuno Reserch). DAPI (Sigma) was used at 0.05 mg/mL. Images were recorded with a Leica Laser Scanning Confocal Microscope.
S-Figure 3. **P1** shows exclusively nuclear localization in the squamous epithelium of the *Drosophila* wing imaginal disc (A) or leg imaginal disc (B), in the columnar epithelium of the antennal imaginal disc (C), in the fat body (D), and in tracheal cells (E). **P2** shows nuclear localization but with weak membrane staining in the fat body (F). **P3** does not stain any of tissues (G, staining of the wing imaginal disc is shown). DAPI shows exclusively nuclear staining in the columnar epithelium of the wing imaginal disc (H).

**Agarose gel electrophoresis**

A 0.8% agarose (w/v) gel in 1X TBE buffer was run for 2 h at 4 V/cm. 5µL 1kb DNA ladder (PeqLab) was incubated with 5 µL 0.2% core-shell nanoparticle for 10 min before loading. The gel was stained with 1 µg/mL ethidium bromide in 1xTBE for 30 min after electrophoresis. The photograph was taken under UV light.

S-Figure 4. Gel-electrophoresis of free DNA (+, -) and a mixture of DNA and **P1** (+, +). **P1** treated DNA fragments migrate at same rate as free DNA, indicating that **P1** does not interact with DNA.
Dot Blotting

10 µL of 1% calf thymus histones solution (Sigma, H7755) was spotted onto a nitrocellulose membrane. 10 µL of 1% BSA was spotted as a control. 2 µL 0.2% Core-shell nanoparticle solution was applied to the spot. After 2 min the membrane was washed with water for 5 min. After drying for 2 min the blot was photographed under UV light.

Vapour diffusion experiment

Samples preparation: P1, 4µL buffer (sigma53245) + 2µL H₂O + 2µL P1 (4mg/ml).
P1/histone complex, 4µL buffer + 2µL histone H1 (8mg/ml) + 2µL P1 (4mg/ml).
Add 1mL buffer in each wells and drop 1 µL sample solution on cover slide (4 dots per slide).
Invert cover slide on the well and seal with GE Baysilone-Paste. Incubate for one week then take images on a fluorescent microscopy.

Binding Experiments with Isothermal Titration Calorimetry

The H1 histone (fraction f1) was purchased from Sigma and used without further purification. Tris-HCl was dissolved in MiliQ water and the pH was corrected using a 1N HCl solution.

Microcalorimetric Measurements. An isothermal titration calorimeter (ITC), purchased from MicroCal Inc., Northampton, MA, was used. The ITC instrument was periodically calibrated electrically with an internal electric heater. In the microcalorimetric titration, a constant 5 µL portion of 113 µM H1 histone solution in 50 mM Tris-HCl buffer (pH 7.4) containing 100 mM NaCl, was successively injected for 54 times into the reaction cell (1.4078 mL) filled with a 1 µM P1 dendrimer solution in the same buffer. The gap between two consecutive injections was 5 minutes, in order to allow the system to reach the equilibrium after each injection. The dilution of the H1 histone solution upon addition to the pure buffer solution placed in the cell was also determined, using the same number of injections and the same concentration employed in the titration experiment. The heats of interaction during each injection were measured by integration of each titration peak using the ORIGIN 7 software (OriginLab Co. Northampton, MA, delivered with the ITC). For each injection, the dilution heats determined in the control experiment were subtracted from the heats obtained in the interaction experiment. The resulting corrected heats of the interaction curve were used for the calculations of the molar enthalpy, equilibrium constant, entropy, and Gibbs free energy of the reaction.
S-Table 1. Thermodynamic parameters of the interaction between P1 and H1 histone in 50 mM Tris-HCl (pH 7.4) buffer + 100 mM NaCl

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<td>P1</td>
<td>7.6</td>
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<td>132</td>
<td>-39.336</td>
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N* = number of histones per one P1

References